from all three hybridomas recognized the activation domain of HIV-1 **rev**.

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=> d his

     (FILE 'HOME' ENTERED AT 08:23:09 ON 27 JUN 2006)

     FILE 'USPATFULL' ENTERED AT 08:23:19 ON 27 JUN 2006
                E AGUILAR-CORDOVA C E/IN
                E CORDOVA C E A/IN
                E AGUILAR CORDOVA C E/IN
L1              8 S E4-E7
                E BELMONT JOHN W/IN
L2              3 S E3
L3              2 S L2 NOT L1
                E HARPER J WADE/IN
L4              1 S E3
L5              0 S L4 NOT L1

     FILE 'WPIDS' ENTERED AT 08:26:29 ON 27 JUN 2006
                E AGUILAR CORDOVA C E/IN
L6              9 S E2-E5
                E BELMONT J W/IN
L7              2 S E3
L8              1 S L7 NOT L6
                E HARPER J W/IN
L9             10 S E3
L10             9 S L9 NOT L6

     FILE 'USPATFULL' ENTERED AT 08:27:55 ON 27 JUN 2006
                E AGUILAR CORDOVA C E/AU

     FILE 'MEDLINE' ENTERED AT 08:28:23 ON 27 JUN 2006
                E AGUILAR CORDOVA C E/AU
L11            77 S E2-E6
L12             5 S L11 AND (TAT OR REV)
                E BELMONT J W/AU
L13           104 S E3 OR E4 OR E6
L14            99 S L13 NOT L11
L15             0 S L14 AND (TAT OR REV)
                E HARPER J W/AU
L16           148 S E3-E5
L17           147 S L16 NOT L11
L18             1 S L17 AND (TAT OR REV)

     FILE 'USPATFULL' ENTERED AT 08:32:37 ON 27 JUN 2006
L19         13995 S (TAT AND REV)
L20           296 S L19 AND TRANSDOMINANT
L21            36 S L20 AND (TAT/CLM AND REV/CLM)
L22             6 S L21 AND AY<1996

     FILE 'MEDLINE' ENTERED AT 08:35:23 ON 27 JUN 2006
L23           657 S (TAT AND REV)
L24            24 S L23 AND (TRANSDOMINANT OR TRANS-DOMINANT)
L25          5692 S REV
L26           118 S L25 AND (TRANSDOMINANT OR TRANS-DOMINANT OR DOMINANT NEGATIVE

=> s Tat
L27          7253 TAT

=> s l27 and (transdominant or trans-dominant or dominant negative or transdominant negative)
              347 TRANSDOMINANT
            96000 TRANS
            93294 DOMINANT
              344 TRANS-DOMINANT
                    (TRANS(W)DOMINANT)
            93294 DOMINANT
           402725 NEGATIVE
            14653 DOMINANT NEGATIVE
                    (DOMINANT(W)NEGATIVE)
              347 TRANSDOMINANT
           402725 NEGATIVE
              122 TRANSDOMINANT NEGATIVE
                    (TRANSDOMINANT(W)NEGATIVE)
L28           117 L27 AND (TRANSDOMINANT OR TRANS-DOMINANT OR DOMINANT NEGATIVE
                  OR TRANSDOMINANT NEGATIVE)

=> d l28,cbib,ab,100-117

L28  ANSWER 100 OF 117     MEDLINE on STN
94267707.     PubMed ID: 8207644.   Construction and characterization of a
     potent HIV-2 Tat transdominant mutant protein. Echetebu C O; Rhim H;
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Medicine, Houston, Texas 77030.) Journal of acquired immune deficiency syndromes, (1994 Jul) Vol. 7, No. 7, pp. 655-64. Journal code: 8812597. ISSN: 0894-9255. Pub. country: United States. Language: English.

AB The human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2) **Tat** proteins **Tat**-1 and **Tat**-2 stimulate transcription of the viral long terminal repeat (LTR) sequences and are required for efficient viral replication. A class of mutant **Tat** proteins, termed "**transdominant** mutants," has been described that possesses relatively low transactivation activity, yet is able to inhibit the function of wild-type **Tat**. These mutant proteins contain a nonfunctional TAR RNA-binding domain but apparently retain a functional activation domain. A potential limitation for therapeutic use of **transdominant** mutants described to date is their low but significant basal level of transactivation for the HIV-1 or HIV-2 LTRs. In order to make an improved **transdominant** mutant, we have constructed **Tat**-2 proteins that contain mutations in four contiguous arginines at residues 81 to 84 in the RNA-binding domain. Using purified proteins and in vitro RNA-binding assays, we verified that these mutant **Tat**-2 proteins are defective for TAR RNA binding. We also verified that these mutant **Tat**-2 proteins bind to a cellular protein kinase in vitro that we have previously shown to bind specifically to the **Tat**-1 and **Tat**-2 activation domain. Using plasmid cotransfection assays, we compared the phenotypes of these mutant **Tat**-2 proteins with the most potent **Tat**-1 **transdominant** mutant described to date. One **Tat**-2 mutant, named "R81-84A," was found to be equivalent to the **Tat**-1 mutant in ability to inhibit wild-type **Tat** transactivation of HIV-1 and HIV-2 LTRs. Moreover, the R81-84A mutant possessed a significantly lower basal level of transactivation than the **Tat**-1 mutant. The R81-84A **Tat**-2 mutant is therefore a promising reagent for future development as an anti-HIV agent. Additionally, our results suggest that wild-type **Tat**-2 transactivation of the HIV-2 LTR is especially sensitive to inhibition by **transdominant** mutants.

L28 ANSWER 101 OF 117 MEDLINE on STN

94149441. PubMed ID: 8106862. Inhibition of human immunodeficiency virus reactivation from latency by a **tat transdominant negative** mutant. Balboni P G; Bozzini R; Zucchini S; Marconi P C; Grossi M P; Caputo A; Manservigi R; Barbanti-Brodano G. (Institute of Microbiology, School of Medicine, University of Ferrara, Italy.) Journal of medical virology, (1993 Dec) Vol. 41, No. 4, pp. 289-95. Journal code: 7705876. ISSN: 0146-6615. Pub. country: United States. Language: English.

AB A BK virus (BKV) expression vector, specific for human cells, was engineered to express antisense human immunodeficiency virus type 1 (HIV-1) **tat** cDNA (**tat**-AS) or a **tat** mutant in cysteine 22 (tat22). Cysteine residues in the cysteine-rich domain of **tat** are necessary for **tat** transactivation of the HIV-1 long terminal repeat (LTR). Both the AS **tat** and the **tat** mutant significantly inhibited transactivation by **tat** when assayed in cells cotransfected with an expression vector where the reporter gene for chloramphenicol acetyl transferase was driven by the HIV-1 LTR. Infection of Jurkat cell clones stably expressing tat22 (Jurkat/tat22) or **tat**-AS (Jurkat/**tat**-AS) with HIV-1 did not show differences in virus titer in comparison to HIV-1-infected control cells. However, in two Jurkat/tat22 cell clones, entrance of HIV-1 into latency was accelerated significantly and reactivation of HIV-1 from latency induced by tumor necrosis factor-alpha (TNF-alpha) or **tat** was blocked. These results suggest that, in a combined and integrated approach to the treatment of acquired immunodeficiency syndrome (AIDS), anti-**tat** genetic therapy could be successfully applied to maintain virus in latency, thereby extending the duration of the asymptomatic phase preceding full-blown AIDS.

L28 ANSWER 102 OF 117 MEDLINE on STN

94107961. PubMed ID: 8280800. The development and testing of retroviral vectors expressing **trans-dominant** mutants of HIV-1 proteins to confer anti-HIV-1 resistance. Liem S E; Ramezani A; Li X; Joshi S. (University of Toronto, Department of Microbiology, Ontario, Canada.) Human gene therapy, (1993 Oct) Vol. 4, No. 5, pp. 625-34. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB **Trans-dominant** mutants of human immunodeficiency virus type 1 (HIV-1) **Tat** and Rev are attractive candidates for use in gene therapy in the treatment of HIV-1 infections because both are essential for viral replication. Retroviral vectors were constructed to allow either **Tat**-inducible or **Tat**- and Rev-inducible expression of **trans-dominant** mutants of **Tat** and Rev. These vectors were used to infect a human CD4+ lymphocyte-derived cell line, MT4. To determine the efficacy of various **Tat** and Rev mutants in inhibiting HIV-1 multiplication, MT4 cells containing mutant-expressing constructs were infected with HIV-1, and the amount of HIV-1 released in the culture medium was measured for up to 30 days. A high level of resistance was observed in cells expressing the double **tat**/rev mutant in a **Tat**-inducible manner.

L28 ANSWER 103 OF 117 MEDLINE on STN

trans-activator (**Tat**): functional domains and the search for **trans-dominant negative** mutants. Arya S K. (Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.) AIDS research and human retroviruses, (1993 Sep) Vol. 9, No. 9, pp. 839-48. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB Human immunodeficiency virus type 2 (HIV-2) trans-activator (**Tat**) is an important trans-regulator of viral gene expression. It differs from the related HIV-1 **Tat** in certain aspects of its structure and function. HIV-2 **Tat** is composed of 130 amino acids versus 86 amino acids for HIV-1 **Tat**. Apart from certain conserved regions, there is little homology between the two Tats. They also differ in their ability to trans-activate HIV-2 and HIV-1 long terminal repeat (LTR)-directed gene expression. As an aid to understanding its mechanism of action, the functional domains important for HIV-2 **Tat** trans-activation of HIV-2 and HIV-1 LTR-directed gene expression were investigated. Like HIV-1 **Tat**, HIV-2 **Tat** contains conserved cysteine- and arginine-rich domains important for its function. However, HIV-2 **Tat** differs from HIV-1 **Tat** in that about 20% of the HIV-2 **Tat** at the amino terminus was not essential for its trans-activation function while HIV-1 **Tat** amino terminus is reportedly a part of its activation domain. Similarly, about 30% of the protein at the carboxy terminus of HIV-2 **Tat** was not essential. A domain critical for HIV-2 **Tat**-mediated trans-activation was located just upstream of the cysteine-rich domain. This segment is predicted to adopt an alpha-helical conformation and also contains acidic amino acid residues; thus, it may resemble amphipathic helix-type activation domains found in some transcriptional factors. A region with predicted hydrophobic alpha-helical character located between the cysteine- and arginine-rich domains was also important for HIV-2 **Tat** function. HIV-2 **Tat** mutants that were analogs of HIV-1 **Tat** **trans-dominant negative** mutants did not display such a phenotype.

L28 ANSWER 104 OF 117 MEDLINE on STN

93276539. PubMed ID: 8389074. Mutagenesis of EIAV **TAT** reveals structural features essential for transcriptional activation and TAR element recognition. Derse D; Newbold S H. (Laboratory of Viral Carcinogenesis, NCI-Frederick Cancer Research and Development Center, Maryland 21702-1201.) Virology, (1993 Jun) Vol. 194, No. 2, pp. 530-6. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Certain members of the lentivirus subfamily of retroviruses encode unique transcriptional activator (**Tat**) proteins that modify the transcription complex after binding to the 5' end of nascent viral mRNA. The **Tat** proteins are modular, containing RNA-binding and activation domains that can be exchanged between different **Tat** proteins or replaced with heterologous protein fragments. While there is considerable sequence conservation among the divergent **Tat** proteins, there are also some structural differences that might be informative. For example, a cluster of basic amino acids in HIV-1 **Tat** is sufficient for RNA binding in vivo and in vitro. The homologous region of EIAV **Tat** is necessary but not sufficient for recognition of its cognate cis-acting RNA element; the entire C-terminal 26 amino acids of EIAV **Tat**, including the basic patch, are required. To better understand the structure-function relationships in EIAV **Tat**, we have generated a battery of expression plasmids encoding insertion, deletion, and missense mutations in the carboxy-terminal region of the **tat** gene. The plasmids were tested for their ability to trans-activate the EIAV promoter or to trans-inhibit a heterologous **Tat** protein. A mutation of a glutamine to an arginine in the cluster of basic residues generated a potent **trans-dominant** inhibitor of both EIAV and HIV-1 **Tat**, indicating that the mutation abolished RNA binding but did not alter the activation domain. Mutations at the extreme C-terminus of EIAV **Tat** impaired both RNA binding and activation domain functions, suggesting effects on secondary or tertiary structure.

L28 ANSWER 105 OF 117 MEDLINE on STN

93267767. PubMed ID: 8388497. Comparison of **trans-dominant** inhibitory mutant human immunodeficiency virus type 1 genes expressed by retroviral vectors in human T lymphocytes. Bahner I; Zhou C; Yu X J; Hao Q L; Guatelli J C; Kohn D B. (Division of Research Immunology/Bone Marrow Transplantation, Childrens Hospital Los Angeles, California.) Journal of virology, (1993 Jun) Vol. 67, No. 6, pp. 3199-207. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB **trans-Dominant** inhibitory mutant versions of the human immunodeficiency virus type 1 (HIV-1) regulatory genes **tat** and rev have previously been described. We have constructed a series of retroviral vectors to transduce these genes and compare their inhibitory activities. The inhibitory activities were measured with transient transfection assays by using a reporter which expresses an HIV-1 gag-Escherichia coli lacZ fusion protein with strict dependence on coexpression of both **tat** and rev. Additionally, the vectors were packaged as amphotropic virions and used to stably transduce human CEM T lymphocytes. The transduced CEM cells were challenged with HIV-1, and the effects of the mutant HIV-1 genes were determined by measuring the levels of HIV-1 p24gag produced. A

trans-activating activity and lacked inhibitory activity. A **tat** gene with a premature stop codon at amino acid 54 (tat54ter) showed moderate **trans-dominant** inhibition of the reporter plasmid but failed to significantly inhibit HIV-1 replication. The M10 rev mutant, with a 2-amino-acid substitution, showed strong **trans-dominant** inhibitory activity both in the reporter plasmid and in the HIV-1 infection assay. The greatest inhibition of HIV-1 growth was seen when M10 was expressed under the transcriptional control of a human cytomegalovirus promoter; slightly less inhibition was achieved when expression of M10 was controlled by the Moloney murine leukemia virus long terminal repeat, and minimal inhibition was seen when the HIV-1 long terminal repeat controlled the M10 gene. These results demonstrate the potential utility of retroviral vectors expressing **trans-dominant** inhibitory mutant HIV-1 genes for gene therapy approaches to AIDS.

L28 ANSWER 106 OF 117 MEDLINE on STN
93267389. PubMed ID: 8496787. Mutational analysis of the amino and carboxy termini of the HIV-2 **Tat** protein. Echetebu C O; Rice A P. (Division of Molecular Virology, Baylor College of Medicine, Houston, Texas 77030.) Journal of acquired immune deficiency syndromes, (1993 Jun) Vol. 6, No. 6, pp. 550-7. Journal code: 8812597. ISSN: 0894-9255. Pub. country: United States. Language: English.
AB The transactivator proteins of HIV-1 and HIV-2, **Tat**-1 and **Tat**-2, are highly homologous in the center of each molecule but are divergent in the amino and carboxy termini. The structure of **Tat**-1 has been extensively characterized by mutagenesis studies, whereas little is as yet known specifically about the structure of **Tat**-2. To characterize the **Tat**-2 protein, we performed a mutational analysis of the amino and carboxy termini of the fully functional first exon (99 residues) of the **Tat**-2 protein. We found that deletion of residues 8 through 33 in the amino terminus drastically reduced transactivation activity, whereas deletion of residues 8 through 47 largely abolished transactivation activity. We also analyzed chimeric proteins in which the amino termini of the **Tat**-1 and **Tat**-2 proteins were exchanged precisely at the first cysteine in the cysteine-rich regions. Both chimeric proteins possessed very low levels of transactivation activity, indicating that the amino termini of **Tat**-1 and **Tat**-2 are not interchangeable. Truncation mutants in the carboxy terminus were analyzed and amino acid 90 at the end of the basic domain was found to be at or near the limit of carboxy residues that can be deleted without abolishing **Tat**-2 function. A **Tat**-2 mutant truncated after residue 84 within the basic domain was found to be a **transdominant** mutant able to inhibit wild-type **Tat**-1 and wild-type **Tat**-2 activities. Additionally, the results of immunoprecipitations suggested that deletions in the **Tat**-2 amino terminus can reduce protein stability.

L28 ANSWER 107 OF 117 MEDLINE on STN
93170669. PubMed ID: 8094701. Extinction of tyrosine aminotransferase gene activity in somatic cell hybrids involves modification and loss of several essential transcriptional activators. Nitsch D; Boshart M; Schutz G. (Division of Molecular Biology of the Cell I, German Cancer Research Center, Heidelberg.) Genes & development, (1993 Feb) Vol. 7, No. 2, pp. 308-19. Journal code: 8711660. ISSN: 0890-9369. Pub. country: United States. Language: English.
AB Extinction is defined as the loss of cell type-specific gene expression that occurs in somatic cell hybrids derived by fusion of cells with dissimilar phenotypes. To explore the basis of this **dominant-negative** regulation, we have studied the activities of the control elements of the liver-specific gene encoding tyrosine aminotransferase (**TAT**) in hepatoma/fibroblast hybrid crosses. We show that extinction in complete somatic cell hybrids is accompanied by the loss of activity of all known cell type-specific control elements of the **TAT** gene. This inactivity is the result of first, lack of expression of genes coding for the transcriptional activators HNF4 and HNF3 beta and HNF3 gamma, which bind to essential elements of the enhancers; and second, loss of in vivo binding and activity of ubiquitous factors to these enhancers, including CREB, which is the target for repression by the tissue-specific extinguisher locus TSE1. Complete extinction of **TAT** gene activity is therefore a multifactorial process affecting all three enhancers controlling liver-specific and hormone-inducible expression. It results from lack of activation, rather than active repression, and involves both post-translational modification and loss of essential transcriptional activators.

L28 ANSWER 108 OF 117 MEDLINE on STN
93028565. PubMed ID: 1409715. Inhibition of human immunodeficiency virus type 1 replication in human T cells by retroviral-mediated gene transfer of a **dominant-negative** Rev trans-activator. Bevec D; Dobrovnik M; Hauber J; Bohnlein E. (Sandoz Research Institute, Vienna, Austria.) Proceedings of the National Academy of Sciences of the United States of America, (1992 Oct 15) Vol. 89, No. 20, pp. 9870-4. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.
AB Human immunodeficiency virus type 1 (HIV-1) is the causative agent of the

treatment for this viral disease is available. Somatic gene therapy has been proposed as an alternative to conventional therapies. Several antiviral gene therapy approaches including ribozymes, antisense inhibition, and RNA-decoy strategies, as well as **dominant-negative** mutants of HIV-1 proteins (Gag, **Tat**, and Rev) have been suggested. To prove the concept of **trans-dominant** inhibition of HIV-1 replication, we transduced CEM cells with a retroviral vector encoding a **dominant-negative** rev gene. Amplification of integrase-specific proviral sequences from high molecular weight DNA indicated successful HIV-1 human T-lymphotropic virus type IIIB (HTLV-IIIB) infection of all cells. In contrast to CEM cells and CEM cells expressing the rev wild-type (wt) gene, infection of two CEM-RevM10 clones with HIV-1 did not result in the release of significant levels of p24 Gag antigen as measured by antigen capture assay, indicating a block in HIV-1 replication due to the presence of the **trans-dominant** Rev protein. Furthermore, the parental CEM cells as well as CEM cells expressing the Rev wt protein were effectively killed in the course of the HIV-1 infection, whereas all CEM cells expressing the RevM10 protein were unaffected in their growth rate.

L28 ANSWER 109 OF 117 MEDLINE on STN
93018835. PubMed ID: 1402661. Stable expression of **transdominant** Rev protein in human T cells inhibits human immunodeficiency virus replication. Malim M H; Freimuth W W; Liu J; Boyle T J; Lyerly H K; Cullen B R; Nabel G J. (Howard Hughes Medical Institute, Ann Arbor, Michigan.) The Journal of experimental medicine, (1992 Oct 1) Vol. 176, No. 4, pp. 1197-201. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB The human immunodeficiency virus (HIV) Rev protein is essential for viral structural protein expression (Gag, Pol, and Env) and, hence, for viral replication. In transient transfection assays, mutant forms of Rev have been identified that inhibit wild-type Rev activity and therefore suppress viral replication. To determine whether such **transdominant** Rev proteins could provide long-term protection against HIV infection without affecting T cell function, T leukemia cell lines were stably transduced with a retroviral vector encoding a **transdominant** mutant of the Rev protein, M10. While all the M10-expressing cell lines remained infectable by HIV-1, these same cells failed to support a productive replication cycle when infected with a cloned isolate of HIV-1. In addition, two out of three M10-expressing CEM clones were also resistant to highly productive infection by a heterogeneous HIV-1 pool. Expression of M10 did not affect induction of HIV transcription mediated by the kappa B regulatory element or **Tat**. Importantly, constitutive expression of Rev M10 did not alter the secretion of interleukin 2 in response to mitogen stimulation of EL-4 and Jurkat cells. The inhibition of HIV infection in cells stably expressing a **transdominant** Rev protein, in the absence of any deleterious effect on T cell function, suggests that such a strategy could provide a therapeutic effect in the T lymphocytes of acquired immunodeficiency syndrome patients.

L28 ANSWER 110 OF 117 MEDLINE on STN
92399512. PubMed ID: 1356022. Cells induced to express a human immunodeficiency virus type 1 envelope gene mutant inhibit the spread of wild-type virus. Buchschacher G L Jr; Freed E O; Panganiban A T. (McArdle Laboratory for Cancer Research, University of Wisconsin, Madison 53706.) Human gene therapy, (1992 Aug) Vol. 3, No. 4, pp. 391-7. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB The feasibility of using a **trans-dominant** interfering human immunodeficiency virus type 1 (HIV-1) envelope mutant for inducible gene therapy of HIV infection was investigated. Genes encoding wild-type or mutant glycoproteins were introduced into CD4+ cells, where they were stably maintained but not expressed until induced. Envelope (env) gene expression was dependent upon the viral regulatory protein **Tat**. Induction of the mutant env resulted in resistance to cytopathic effects mediated by wild-type envelope and decreased infectious vector virus production. When cells containing the mutant env gene were infected with wild-type virus, viral spread was inhibited. The fact that maintenance of the env gene was stable over time suggests that inducible gene therapy using the dominantly interfering env mutant may be a feasible approach to slowing the progression of HIV-1 disease.

L28 ANSWER 111 OF 117 MEDLINE on STN
92031277. PubMed ID: 1931822. **Trans-dominant Tat** mutants with alterations in the basic domain inhibit HIV-1 gene expression. Modesti N; Garcia J; Debouck C; Peterlin M; Gaynor R. (Division of Hematology-Oncology, UCLA School of Medicine 94143.) The New biologist, (1991 Aug) Vol. 3, No. 8, pp. 759-68. Journal code: 9000976. ISSN: 1043-4674. Pub. country: United States. Language: English.

AB The **Tat** protein of the human immunodeficiency virus type 1 (HIV-1) is required for efficient viral gene expression. By means of mutational analyses, several domains of the **Tat** protein that are required for complete activation of HIV-1 gene expression have been defined. These include an amino-terminal activating domain, a cysteine-rich dimerization

trans-activation response element (TAR) and in **Tat** nuclear localization. Recently, we described a mutation, known as delta **tat**, which resulted in a protein with a truncated basic domain. This protein had a "**trans-dominant**" phenotype in that it inhibited wild-type **Tat** activation of the HIV-1 LTR. To further characterize the requirements for generating a **Tat trans-dominant** phenotype, we constructed a variety of **Tat** proteins with truncations or substitutions in the basic domain. A number of these proteins showed a **trans-dominant** phenotype. These **Tat** mutants also inhibited activation of the HIV-1 LTR by a protein composed of **Tat** fused to the prokaryotic R17 (phage MS2) RNA-binding protein in which the R17 recognition element was inserted in the HIV-1 LTR in place of TAR. Thus, an intact TAR element was not required for this inhibition. We also studied the cellular localization of **Tat** and a **trans-dominant Tat** mutant by means of immunofluorescence staining with the use of antibodies reactive to different domains of the **Tat** protein. The results indicated that **Tat** becomes localized predominantly in the nucleus both in the presence and absence of the **trans-dominant Tat** construct, suggesting that the **trans-dominant** mutant does not inhibit **Tat** nuclear localization. These studies further define the requirements for the creation of **trans-dominant Tat** mutants, and suggest that the mechanism of **trans-dominant Tat** inhibition may be either the formation of an inactive complex between wild-type and mutant **Tat** or sequestration of cellular factors involved in regulating HIV-1 gene expression.

L28 ANSWER 112 OF 117 MEDLINE on STN
91303679. PubMed ID: 2072452. Mutational definition of the human immunodeficiency virus type 1 Rev activation domain. Malim M H; McCarn D F; Tiley L S; Cullen B R. (Howard Hughes Medical Institute, Department of Medicine, Duke University Medical Center, Durham, North Carolina 27710.) Journal of virology, (1991 Aug) Vol. 65, No. 8, pp. 4248-54. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Replication of human immunodeficiency virus type 1 requires the functional expression of the virally encoded Rev protein. The binding of this nuclear trans activator to its viral target sequence, the Rev-response element, induces the cytoplasmic expression of unspliced viral mRNAs. Mutation of the activation domain of Rev generates inactive proteins with normal RNA binding capabilities that inhibit wild-type Rev function in a **trans-dominant** manner. Here, we report that the activation domain comprises a minimum of nine amino acids, four of which are critically spaced leucines. The preservation of this essential sequence in other primate and nonprimate lentivirus Rev proteins indicates that this leucine-rich motif has been highly conserved during evolution. This conclusion, taken together with the observed permissiveness of a variety of eukaryotic cell types for Rev function, suggests that the target for the activation domain of Rev is likely to be a highly conserved cellular protein(s) intrinsic to nuclear mRNA transport or splicing.

L28 ANSWER 113 OF 117 MEDLINE on STN
90311346. PubMed ID: 2195547. A **transdominant tat** mutant that inhibits **tat**-induced gene expression from the human immunodeficiency virus long terminal repeat. Pearson L; Garcia J; Wu F; Modesti N; Nelson J; Gaynor R. (Department of Medicine, University of California-Los Angeles School of Medicine 90024.) Proceedings of the National Academy of Sciences of the United States of America, (1990 Jul) Vol. 87, No. 13, pp. 5079-83. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Regulation of human immunodeficiency virus (HIV) gene expression is dependent on specific regulatory regions in the long terminal repeat. These regions include the enhancer, SP1, "TATA," and trans-activating (TAR) regions. In addition, viral regulatory proteins such as **tat** and rev are important in regulating HIV gene expression. The mechanism of **tat** activation remains the subject of investigation, but effects at both transcriptional and posttranscriptional levels seem likely. Previous mutagenesis of the **tat** protein revealed that the amino terminus, the cysteine-rich domain, and the basic domain were all required for complete **tat** activation. Mutants of other viral trans-acting regulatory proteins, including E1A, tax, and VM65, have been identified that were capable of antagonizing the activity of their corresponding wild-type proteins. We wished to determine whether mutants of the **tat** protein could be identified that exhibited a similar phenotype. One mutant (delta **tat**) that truncated the basic domain of **tat** resulted in a **transdominant** phenotype inhibiting **tat**-induced gene expression of the HIV long terminal repeat but not other viral promoters. This mutant exhibited its maximal phenotype in cotransfection experiments when present in an 8- to 30-fold molar excess over the wild-type **tat** gene. Trans-activation of the HIV long terminal repeat by delta **tat** was very defective at the DNA concentrations used in these experiments. RNase protection analysis indicated that this mutant decreased **tat**-induced steady-state mRNA levels of the HIV long terminal repeat. Second-site mutations of the delta **tat** gene in either the amino terminus or cysteine

which was localized predominantly to the nucleolus, delta **tat** was present in both the nucleus and cytoplasm, suggesting that it may inhibit **tat** function by preventing nucleolar localization. **Transdominant** mutants of **tat** may have a role in potentially inhibiting HIV gene expression.

L28 ANSWER 114 OF 117 MEDLINE on STN

90263116. PubMed ID: 1971524. A cyclic AMP response element mediates repression of tyrosine aminotransferase gene transcription by the tissue-specific extinguisher locus Tse-1. Boshart M; Weih F; Schmidt A; Fournier R E; Schutz G. (Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg.) Cell, (1990 Jun 1) Vol. 61, No. 5, pp. 905-16. Journal code: 0413066. ISSN: 0092-8674. Pub. country: United States. Language: English.

AB Tyrosine aminotransferase (**TAT**) gene expression is liver specific and inducible by glucocorticoids and via the cAMP signaling pathway. In fibroblasts and other nonliver cells the gene is subject to negative control by the **trans-dominant** tissue-specific extinguisher locus Tse-1. We identified a hepatocyte-specific enhancer that is repressed by Tse-1. Two distinct sequence motifs are absolutely essential for function of this enhancer: a cAMP response element (CRE), which is the target for repression by Tse-1, and a hepatocyte-specific element. The specificity of the enhancer is generated by the combination of these two essential elements, which are fully interdependent. In vivo footprinting indicates that Tse-1 acts by affecting protein binding at the CRE. A direct antagonism between Tse-1 and the cAMP signaling pathway suggests that Tse-1 plays a role in control of developmental activation of the **TAT** gene.

L28 ANSWER 115 OF 117 MEDLINE on STN

90160317. PubMed ID: 2137611. Silencing of human immunodeficiency virus long terminal repeat expression by an adenovirus E1a mutant. Ventura A M; Arens M Q; Srinivasan A; Chinnadurai G. (Institute for Molecular Virology, Saint Louis University School of Medicine, MO 63110.) Proceedings of the National Academy of Sciences of the United States of America, (1990 Feb) Vol. 87, No. 4, pp. 1310-4. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Gene expression from the human immunodeficiency virus (HIV) long terminal repeat (LTR) is strongly stimulated by the viral **tat** gene. The HIV LTR is also activated by several physical and chemical agents and heterologous viral genes, including adenovirus E1a. As E1a has separable transcriptional activation and repression functions, we examined the negative regulatory effects of E1a on the expression of the HIV LTR by using a **trans-dominant** E1a mutant. Mutant hr5 strongly suppressed the basal activity of the LTR as well as trans-activation of the LTR by heterologous agents such as the cytomegalovirus immediate early gene or DNA-damaging agents such as mitomycin C and UV irradiation. In addition, hr5 also caused significant suppression of **tat** gene-mediated trans-activation. The suppression of HIV LTR expression by hr5 appears to be mediated, at least in part, by the repression of the HIV enhancer, as the activity of an enhancer test system composed of the human T-cell leukemia virus I LTR containing an HIV-1 enhancer substitution was severely repressed by hr5. Cotransfection of HIV-1 proviral DNA with hr5 DNA resulted in a significant reduction of HIV production.

L28 ANSWER 116 OF 117 MEDLINE on STN

89324055. PubMed ID: 2752420. Mutational analysis of HIV-1 **Tat** minimal domain peptides: identification of **trans-dominant** mutants that suppress HIV-LTR-driven gene expression. Green M; Ishino M; Loewenstein P M. (Institute for Molecular Virology, St. Louis University School of Medicine, Missouri 63110.) Cell, (1989 Jul 14) Vol. 58, No. 1, pp. 215-23. Journal code: 0413066. ISSN: 0092-8674. Pub. country: United States. Language: English.

AB The HIV-1 **Tat** protein is a potent trans-activator essential for virus replication. We reported previously that HIV-1 **Tat** peptides containing residues 37-48 (mainly region II), a possible activating region, and residues 49-57 (region III), a nuclear targeting and putative nucleic acid binding region, possess minimal but distinct trans-activator activity. The presence of residues 58-72 (region IV) greatly enhances trans-activation. We postulate that **Tat** mutant peptides with an inactive region II and a functional region III can behave as **dominant negative** mutants. We synthesized minimal domain peptides containing single amino substitutions for amino acid residues within region II that are conserved among different HIV isolates. We identify four amino acid residues whose substitution within **Tat** minimal domain peptides leads to defects in transactivation. Some of these mutants are **trans-dominant** in several peptide backbones, since they strongly inhibit trans-activation by wild-type **Tat** protein added to cells or expressed from microinjected plasmid. Significantly, trans-activation of integrated HIV-LTRCAT is blocked by some **trans-dominant** mutant peptides. These results suggest an attractive approach for the development of an AIDS therapy.

L28 ANSWER 117 OF 117 MEDLINE on STN

trans-dominant loci regulate expression of liver-specific traits in hepatoma hybrid cells. Killary A M; Fournier R E. Cell, (1984 Sep) Vol. 38, No. 2, pp. 523-34. Journal code: 0413066. ISSN: 0092-8674. Pub. country: United States. Language: English.

AB Extinction is an operational term that refers to the lack of expression of tissue-specific traits that is generally observed in hybrid cells formed by fusing dissimilar cell types. To define the genetic basis of this phenomenon, a series of rat hepatoma x mouse fibroblast hybrids has been isolated and characterized. We report here that the extinction of hepatic marker traits in these clones was strictly correlated with the retention of five particular fibroblast chromosomes (autosomes 8, 9, 10, 11, and 13). In order to dissect this correlation into its component parts, hepatoma microcell hybrids containing single, specific fibroblast chromosomes were constructed. Hepatoma clones retaining only fibroblast chromosome 11 were specifically extinguished for liver-specific tyrosine aminotransferase (**TAT**) expression, while expression of four other hepatic traits and of numerous constitutive markers was unaffected. Furthermore, removal of fibroblast chromosome 11 from the populations by back-selection resulted in reexpression of **TAT** activity to full parental levels. These data define and localize a genetic locus, tissue-specific extinguisher-1 (Tse-1), which regulates hepatic **TAT** expression in trans. We also provide evidence that human Tse-1 resides on the homologous chromosome (human chromosome 17), and that hybrids retaining active Tse-1 loci lack **TAT**-specific mRNA.

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L28 ANSWER 75 OF 117 MEDLINE on STN

1998333918. PubMed ID: 9670843. Regulation of TNFalpha and TGFbeta-1 gene transcription by HIV-1 **Tat** in CNS cells. Sawaya B E; Thatikunta P; Denisova L; Brady J; Khalili K; Amini S. (Center for NeuroVirology and NeuroOncology, Department of Neurology, Allegheny University of Health Sciences, Philadelphia, PA 19102, USA.) Journal of neuroimmunology, (1998 Jul 1) Vol. 87, No. 1-2, pp. 33-42. Journal code: 8109498. ISSN: 0165-5728. Pub. country: Netherlands. Language: English.

AB **Tat** is a transcription transactivator produced by the human immunodeficiency virus type 1 (HIV-1) at the early phase of infection and plays a critical role in the expression and replication of the viral genome. This 86 amino acid protein, which can be secreted from the infected cells, has the ability to enter uninfected cells and exert its activity upon the responsive genes. Earlier results indicated that in addition to the HIV-1 promoter, **Tat** has the capacity to induce transcription of a variety of cellular genes. In this study, we demonstrate that exposure of cells from the central nervous system (U-87MG and SK-N-MC) and the lymphoid T cells (Jurkat) to highly purified **Tat** increases transcriptional activity of the reporter constructs containing the promoters from the transforming growth factor beta-1 (TGFbeta-1), the tumor necrosis factor alpha (TNFalpha), and the HIV-1 LTR. In addition, **Tat** treatment results in increased levels of TGFbeta-1 and TNFalpha mRNAs in these cells. Activation of the TGFbeta-1 and TNFalpha promoter constructs by **Tat** in U-87MG and SK-N-MC cells required amino acid residues 2 to 36 which spans the acidic and the cysteine-rich domains of **Tat**. In both CNS and lymphoid cells, the level of endogenous TGFbeta-1 mRNA was increased by mutant **Tat** protein containing amino acids 1 to 48 but not with a mutant **Tat** protein with a deletion between residues 2 to 36. TNFalpha mRNA level was increased by mutant **Tat** spanning residues 1 to 48 in U-87MG cells, but not in SK-N-MC and Jurkat cells. These observations suggest that activation of cellular and viral genes by **Tat** in various cells may be mediated by different pathways as evidenced by the requirements of the different regions of **Tat**. Activation of the TGFbeta-1 and TNFalpha promoters by wild-type **Tat** was severely affected by the mutant peptides spanning residues 2 to 36 and 1 to 48 suggesting that both truncated **Tat** peptides may function as **dominant negative** mutants over TNFalpha and TGFbeta-1 gene transcription. The importance of these findings in **Tat**-induced regulation of viral and cellular genes in various cell types is discussed.

L28 ANSWER 76 OF 117 MEDLINE on STN

1998197329. PubMed ID: 9536266. Inhibition of HIV-1 replication by combined expression of gag **dominant negative** mutant and a human ribonuclease in a tightly controlled HIV-1 inducible vector. Cara A; Rybak S M; Newton D L; Crowley R; Rottschafer S E; Reitz M S Jr; Gusella G L. (Basic Research Laboratory, NCI, NIH, Bethesda, MD, USA.) Gene therapy, (1998 Jan) Vol. 5, No. 1, pp. 65-75. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB An HIV-1-based expression vector has been constructed that produces protective genes tightly regulated by HIV-1 **Tat** and Rev proteins. The vector contains either a single protective gene (HIV-1 gag **dominant negative** mutant (delta-gag)) or a combination of two different protective genes (delta-gag and eosinophil-derived neurotoxin (EDN), a human ribonuclease) which are expressed from a dicistronic mRNA. After

viral production was completely inhibited in cells transduced with the vector producing both delta-gag and EDN and delayed in cells producing delta-gag alone. In addition, cotransfection of HeLa-**Tat** cells with an infectious HIV-1 molecular clone and either protective vector demonstrated that the HIV-1 packaging signals present in the constructs were functional and allowed the efficient assembly of the protective RNAs into HIV-1 virions, thus potentially transmitting protection to the HIV-1 target cells.

L28 ANSWER 77 OF 117 MEDLINE on STN
1998184222. PubMed ID: 9525310. Inhibition of human immunodeficiency virus replication and growth advantage of CD4+ T cells from HIV-infected individuals that express intracellular antibodies against HIV-1 gp120 or **Tat**. Poznansky M C; Foxall R; Mhashilkar A; Coker R; Jones S; Ramstedt U; Marasco W. (The Dept. of Genito-Urinary Medicine and Communicable Diseases, Imperial College School of Medicine at St. Mary's, London, UK.) Human gene therapy, (1998 Mar 1) Vol. 9, No. 4, pp. 487-96. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.
AB Current clinical gene therapy protocols for the treatment of human immunodeficiency virus type 1 (HIV-1) infection often involve the ex vivo transduction and expansion of CD4+ T cells derived from HIV-positive patients at a late stage in their disease (CD4 count <400). These protocols involve the transduction of T cells by murine leukemia virus (MLV)-based vectors encoding antiviral constructs such as the rev m10 **dominant negative** mutant or a ribozyme directed against the CAP site of HIV-1 RNA. We examined the efficiency and stability of transduction of CD4+ T cells derived from HIV-infected patients at different stages in the progression of their disease, from seroconversion to AIDS. CD4+ T cells from HIV-positive patients and uninfected donors were transduced with MLV-based vectors encoding beta-galactosidase and an intracellular antibody directed against gp120 (sFv 105) or **Tat**. (sFvtat1-Ckappa). The expression of marker genes and the effects of the antiviral constructs were monitored in vitro in unselected transduced CD4+ T cells. Efficiency and stability of transduction varied during the course of HIV infection; CD4+ T cells derived from asymptomatic patients were transducible at higher efficiencies and stabilities than CD4+ T cells from patients with acquired immunodeficiency syndrome (AIDS). Expression of the anti-**tat** intracellular antibody was more effective at stably inhibiting HIV-1 replication in transduced cells from HIV-infected individuals than was sFv 105. The results of this study have important implications for the development of a clinically relevant gene therapy for the treatment of HIV-1 infection.

L28 ANSWER 78 OF 117 MEDLINE on STN
1998139870. PubMed ID: 9473483. Modulation of HTLV-I gene expression by HIV-1 Rev through an alternative RxRE-independent pathway mediated by the RU5 portion of the 5'-LTR. Kubota S; Furuta R A; Hatanaka M; Pomerantz R J. (Dorrance H. Hamilton Laboratories, Department of Medicine, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107, USA.) Biochemical and biophysical research communications, (1998 Feb 4) Vol. 243, No. 1, pp. 79-85. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.
AB The 5'-RU5 portion of human T-lymphocyte virus type I (HTLV-I) long terminal repeat (LTR) had been reported to contain cis-acting elements for the controlled viral gene expression by the rex gene product. In this study, the human immunodeficiency virus type I (HIV-1) Rev protein was found to enhance gene expression, acting through the 5'-RU5 portion of HTLV-I, while the Rex-responsive element (RxRE)-mediated activation by Rev was reconfirmed to be negative. This positive action of HIV-1 Rev on HTLV-I gene expression seemed to be distinct from the widely accepted Rex or Rev function to facilitate the nuclear export of RxRE-containing unspliced viral mRNAs, since a **trans-dominant**, nuclear export-deficient mutant (RevM10) still retained the RU5-mediated effector function. Analyses of the functional aspects of **Tat**/Rev fusion proteins on the HTLV-I RU5 suggested a specific interaction of Rev and RU5, but lacked evidence for the binding of Rev to the RU5 at the RNA level. These results suggest an answer to the controversy regarding a Rex-like function occasionally observed with HIV-1 Rev and its related proteins. It may also be suggested that particular care should be taken when such a **trans-dominant** Rev mutant is considered to be used as a genetic therapy against HIV-I infection, in individuals infected with both HIV-I and HTLV-1.

L28 ANSWER 79 OF 117 MEDLINE on STN
1998139081. PubMed ID: 9499041. Comparative analyses of intracellularly expressed antisense RNAs as inhibitors of human immunodeficiency virus type 1 replication. Veres G; Junker U; Baker J; Barske C; Kalfoglou C; Ilves H; Escaich S; Kaneshima H; Bohnlein E. (Systemix Inc., a Novartis Company, Palo Alto, California 94304, USA.. gveres@stem.com) . Journal of virology, (1998 Mar) Vol. 72, No. 3, pp. 1894-901. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB The antiviral activities of intracellularly expressed antisense RNAs

and env genes and the 3' long terminal repeat (LTR) sequence were evaluated in this comparative study. Retroviral vectors expressing the antisense RNAs as part of the Moloney murine leukemia virus LTR promoter-directed retroviral transcript were constructed. The CD4+ T-cell line CEM-SS was transduced with retroviral constructs, and Northern blot analyses showed high steady-state antisense RNA expression levels. The most efficient inhibition of HIV-1 replication was observed with the env antisense RNA, followed by the pol complementary sequence, leading to 2- to 3-log10 reductions in p24 antigen production even at high inoculation doses (4 x 10(4) 50% tissue culture infective doses) of the HIV-1 strain HXB3. The strong antiviral effect correlated with a reduction of HIV-1 steady-state RNA levels, and with intracellular **Tat** protein production, suggesting that antisense transcripts act at an early step of HIV-1 replication. A lower steady-state antisense RNA level was detected in transduced primary CD4+ lymphocytes than in CEM-SS cells. Nevertheless, replication of the HIV-1 JR-CSF isolate was reduced with both the pol and env antisense RNA. Intracellularly expressed antisense sequences demonstrated more pronounced antiviral efficacy than the **transdominant** RevM10 protein, making these antisense RNAs a promising gene therapy strategy for HIV-1.

L28 ANSWER 80 OF 117 MEDLINE on STN

1998086588. PubMed ID: 9425451. Inhibition of HIV-1 replication by a **Tat transdominant negative** mutant in human peripheral blood lymphocytes from healthy donors and HIV-1-infected patients. Rossi C; Balboni P G; Betti M; Marconi P C; Bozzini R; Grossi M P; Barbanti-Brodano G; Caputo A. (Department of Experimental and Diagnostic Medicine, University of Ferrara, Italy.) Gene therapy, (1997 Nov) Vol. 4, No. 11, pp. 1261-9. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB It was previously shown that a **tat** mutant (tat22) where cysteine 22 is substituted by glycine behaves as a **transdominant negative** mutant in Jurkat T cells lytically or latently infected by HIV-1. In this study we demonstrate that tat22 controls HIV-1 replication in primary cells. This effect was observed both after in vitro infection of peripheral blood mononuclear cells (PBMCs) from normal donors and after reactivation of the latent infection in PBMCs from seropositive patients. The antiviral effect of tat22 was limited to conditions of low virus production. The use of tat22 may be promising for a gene therapy approach to AIDS during the asymptomatic phase of the disease allowing control of virus replication in infected cells and inhibition of virus spread to uninfected cells.

L28 ANSWER 81 OF 117 MEDLINE on STN

97445047. PubMed ID: 9300036. Distinct transcriptional pathways of TAR-dependent and TAR-independent human immunodeficiency virus type-1 transactivation by **Tat**. Yang L; Morris G F; Lockyer J M; Lu M; Wang Z; Morris C B. (School of Biology, Georgia Institute of Technology, Atlanta 30332, USA.) Virology, (1997 Aug 18) Vol. 235, No. 1, pp. 48-64. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB **Tat** stimulates HIV-1 gene expression during transcription initiation and elongation. **Tat** functions primarily through specific interactions with TAR RNA and several putative cellular cofactors to increase the processivity of RNA polymerase II complexes during HIV-1 transcription elongation. Although HIV-1 transactivation by **Tat** in most cell types requires intact TAR sequences, previous reports demonstrate that **Tat** transactivates HIV-1 long terminal repeat (LTR)-directed gene expression in several central nervous system-derived astrocytic/glial cell lines in the absence of TAR. Within this study, transient expression assays performed in the astrocytic/glial cell line, U87-MG, confirm that kappa B elements within the HIV-1 LTR mediate TAR-independent transactivation by **Tat** and demonstrate additionally that distinct amino acid residues within the cysteine-rich activation domain of **Tat** are required for TAR-independent versus TAR-dependent transactivation. Established U87-MG cell lines expressing a **transdominant negative** mutant of I kappa B alpha, I kappa B alpha delta N, fail to support TAR-independent transactivation by **Tat**, suggesting that binding of NF-kappa B to kappa B enhancer elements within the HIV-1 LTR is necessary for **Tat**-mediated transactivation in the absence of TAR. Ribonucleic acid protection analyses of promoter-proximal and -distal transcripts derived from TAR-deleted and TAR-containing HIV-1 LTR reporter constructs in U87-MG cells indicate that the predominant effect of **Tat** during TAR-independent transactivation occurs at the lavel of transcription initiation, whereas a prominent elongation effect of **Tat** is observed in the presence of TAR. These data suggest an alternative regulatory pathway for **Tat** transactivation in specific cells derived from the central nervous system that is independent of TAR and that requires direct or indirect interaction of **Tat** with NF-kappa B-binding sites in the HIV-1 LTR.

L28 ANSWER 82 OF 117 MEDLINE on STN

97437478. PubMed ID: 9292011. Biochemical and functional interaction of

general transcription factor TFIIB. Veschambre P; Roisin A; Jalinot P. (Laboratoire de Biologie Moleculaire et Cellulaire, CNRS UMR49, Ecole Normale Superieure de Lyon, France.) The Journal of general virology, (1997 Sep) Vol. 78 (Pt 9), pp. 2235-45. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **Tat** strongly stimulates transcription of the human immunodeficiency type 1 (HIV-1) provirus by interacting with various cellular transcription factors, including TFIID. The results presented in this report indicate that the effect exerted by **Tat** also involves an interaction with TFIIB. A direct protein-protein interaction between **Tat** and TFIIB was observed in vitro. Detailed analysis of this interaction showed that the cysteine-rich and core domains of **Tat** bind to the N-terminal moiety of the general transcription factor. The role of the interaction between **Tat** and TFIIB in the activation of the entire HIV-1 promoter was analysed. Transfection experiments performed using a reporter construct containing the HIV-1 long terminal repeat fused to a reporter gene showed that overexpression of TFIIB progressively suppressed **Tat**-induced transcription. This effect was weakened by an increase in the intracellular concentration of **Tat**. A similar consequence of TFIIB overexpression was observed in a HeLa cell line stably transformed with a construct corresponding to the lacZ gene under the control of the HIV-1 promoter. Mutants of TFIIB which differed in their ability to interact with **Tat** and to function in basal transcription were analysed. The ability of TFIIB mutants defective for basal transcription to inhibit **Tat**-induced activity of the HIV-1 promoter depended on their capacity to interact with **Tat**. Mutants of TFIIB functional for basal transcription, but defective for the interaction with **Tat**, exhibited a **dominant negative** effect. From these data we propose a model in which interaction between **Tat** and both general transcription factors TBP and TFIIB maintains the transcriptional initiation complex in an active configuration.

L28 ANSWER 83 OF 117 MEDLINE on STN

97333609. PubMed ID: 9189769. Defective HIV-1 provirus encoding a multitarget-ribozyme inhibits accumulation of spliced and unspliced HIV-1 mRNAs, reduces infectivity of viral progeny, and protects the cells from pathogenesis. Paik S Y; Banerjea A; Chen C J; Ye Z; Harmison G G; Schubert M. (Molecular and Viral Genetics Section, LMMN, National Institute of Neurological Disorder and Stroke, NIH, Bethesda, MD 20892-4164, USA.) Human gene therapy, (1997 Jun 10) Vol. 8, No. 9, pp. 1115-24. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB A HeLa T4 cell line containing a defective human immunodeficiency virus type 1 (HIV-1) DNA (HD4) was isolated. After transactivation with **Tat**, the HD4 DNA was transcribed into a single 3.7-kb mRNA that encodes a chimeric CD4/Env protein and a multitarget-ribozyme directed against multiple sites within the gp120 coding region of HIV-1 RNA (Chen et al., 1992). Early steps in HIV infection such as entry, reverse transcription, and proviral DNA formation were not affected in HD4 cells, and HD4 was efficiently transactivated after either HIV-1 or HIV-2 infections. HIV-2, which lacks all of the HIV-1-specific ribozyme target sites, replicated to high levels in HD4 cells whereas HIV-1 replication was selectively inhibited. Despite a reduced accumulation of all HIV-1 transcripts, transactivation of HD4 was efficient. Surprisingly, the most abundant, multiply spliced mRNAs were reduced even though they lack all of the ribozyme target sites. These results strongly suggest that the ribozyme co-localizes with unspliced HIV-1 pre-mRNA and/or genomic HIV-1 RNA in the nucleus. Cleavage of these precursor RNAs explains the reduction of all spliced and unspliced HIV-1 RNAs. Cleavage of genomic RNA probably contributed to the three-fold reduction in the infectivity of viral progeny. Thus, the HD4 ribozyme RNA functioned as a ribozyme in the nucleus and as a mRNA for a chimeric CD4/Env protein in the cytoplasm. Its unusual large size for a ribozyme (3.7 kb) indicates that, in the future, other antiviral proteins, like negative **transdominant** mutant HIV-1 proteins, may also be encoded to increase its antiviral potential in a gene therapy approach.

L28 ANSWER 84 OF 117 MEDLINE on STN

97319610. PubMed ID: 9176513. Studies on the effect of the combined expression of anti-**tat** and anti-rev genes on HIV-1 replication. Caputo A; Rossi C; Bozzini R; Betti M; Grossi M P; Barbanti-Brodano G; Balboni P G. (Institute of Microbiology, School of Medicine, University of Ferrara, Italy.) Gene therapy, (1997 Apr) Vol. 4, No. 4, pp. 288-95. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A series of retroviral vectors with potential anti-**tat** and antirev activity was developed. Vectors containing a **tat transdominant negative** mutant (tat22/37) and an RRE decoy in different positions, directed by the same promoter or by different promoters, were generated. Retroviral vectors containing tat22/37 and the RevM10 **transdominant negative** mutant were also constructed. Jurkat cells were transduced with the recombinant retroviruses to produce monoclonal and polyclonal

integrated and expression of the inserted genes was detected by Northern blot or RT-PCR analysis. However, infection of these cell lines with HIV-1 showed that none of these recombinant constructs inhibited virus replication at a high multiplicity of infection (MOI). At a low MOI, two cell clones containing tat22/37 and the RRE decoy in 3' position showed a long lasting protection against virus replication, in comparison to control cultures expressing tat22/37 or RRE alone. Combination of **tat** and rev mutants was ineffective in inhibiting HIV-1 replication at both low and high MOIs. At a low MOI, HIV-1 replication was efficiently blocked in two cell clones expressing the RevM10 mutant alone. These results show a synergic effect of anti-**tat** and anti-rev molecules when the RRE sequence is cloned 3' to tat22/37, suggesting the possibility of using this vector design to control HIV-1 replication.

L28 ANSWER 85 OF 117 MEDLINE on STN

97288989. PubMed ID: 9143912. Protection of primary human T cells from HIV infection by Trev: a **transdominant** fusion gene. Chinen J; Aguilar-Cordova E; Ng-Tang D; Lewis D E; Belmont J W. (Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030, USA.) Human gene therapy, (1997 May 1) Vol. 8, No. 7, pp. 861-8. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB Gene therapy is one of several approaches that are being tested in the search for an effective anti-human immunodeficiency virus (HIV) treatment. In this strategy, a "protective" gene would be introduced into target cells, rendering them relatively resistance to the virus-induced cytopathicity. **Tat** and Rev are viral proteins essential for HIV gene expression. **Tat** increases viral gene transcription and Rev is responsible for the nuclear export of mRNA encoding structural viral proteins. A fusion protein (Trev) was constructed, joining **Tat** and Rev **transdominant** mutant gene sequences. Previously, we showed that Trev inhibits both **Tat** and Rev activities in Jurkat T cells. To determine whether Trev could inhibit HIV replication in primary cells, we transferred the trev gene to peripheral blood lymphocytes and challenged them with different HIV strains. Levels of HIV p24 antigen (Ag) were reduced 4- to 15-fold in cultures of Trev-CD4+ T cells infected with two HIV primary clinical isolates and were not detectable in cultures infected with HIV strains NL4-3 and SF2. In contrast, cultures of nontransduced CD4+ T cells infected with the same viruses had levels of HIV p24 Ag up to 10 ng/ml. Trev-transduced CD4+ T cells demonstrated increased survival following HIV challenge for the length of the experiments (30 days). We did not observe rapid emergence of Trev-resistant HIV in our cultures. Following HIV challenge, cell-associated Trev protein was increased, supporting the hypothesis that cells surviving Trev expression provided a cell survival advantage. This work showed that Trev was able to inhibit HIV replication in primary CD4+ T cells, and, therefore the trev gene could be a candidate for gene therapy against HIV.

L28 ANSWER 86 OF 117 MEDLINE on STN

97234667. PubMed ID: 9116267. Inhibition of human immunodeficiency virus-1 (HIV-1) replication after transduction of granulocyte colony-stimulating factor-mobilized CD34+ cells from HIV-1-infected donors using retroviral vectors containing anti-HIV-1 genes. Bauer G; Valdez P; Kearns K; Bahner I; Wen S F; Zaia J A; Kohn D B. (Department of Pediatrics, University of Southern California School of Medicine, Los Angeles, USA.) Blood, (1997 Apr 1) Vol. 89, No. 7, pp. 2259-67. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Transfer of "anti-HIV-1 genes" into hematopoietic stem cells of human immunodeficiency virus-1 (HIV-1)-infected individuals may be a potent therapeutic approach to render mature cells arising from transduced stem cells resistant to the destructive events associated with HIV-1 infection. To determine the feasibility of gene therapy for acquired immunodeficiency syndrome in individuals already infected with HIV-1, granulocyte colony-stimulating factor mobilized peripheral blood CD34+ cells were isolated from HIV-1-infected individuals and transduced with retroviral vectors containing three different anti-HIV-1-genes: the Rev binding domain of the Rev Responsive Element (RRE decoy) (L-RRE-neo), a double hammerhead ribozyme vector targeted to cleave the **tat** and rev transcripts (L-TR/**TAT**-neo), and the **trans-dominant** mutant of rev (M10) (L-M10-SN). As a control, a vector mediating only neomycin resistance (LN) was used. After 3 days of transduction on allogeneic stroma in the presence of stem cell factor, interleukin-6 (IL-6), and IL-3, the cultures were G418-selected, and then challenged with HIV-1(JR-FL) and a primary HIV-1 isolate. Compared with the control cultures, the L-RRE-neo-, L-TR/**TAT**-neo-, and L-M10-SN-transduced cultures displayed up to 1,000-fold inhibition of HIV-1 replication after challenge with HIV-1(JR-FL) and the primary HIV-1 isolate. Growth of the hematopoietic cells in long-term bone marrow culture was not perturbed by the presence of any of the anti-HIV-1 genes. This study shows that anti-HIV-1 genes can be introduced into CD34+ cells from individuals already infected with HIV-1, and strongly inhibit HIV-1 replication in primary monocytes derived from the CD34+ progenitors.

L28 ANSWER 87 OF 117 MEDLINE on STN

97165870. PubMed ID: 9013763. Mutant and wild-type androgen receptors exhibit cross-talk on androgen-, glucocorticoid-, and progesterone-mediated transcription. Yen P M; Liu Y; Palvimo J J; Trifiro M; Whang J; Pinsky L; Janne O A; Chin W W. (Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115, USA.) Molecular endocrinology (Baltimore, Md.), (1997 Feb) Vol. 11, No. 2, pp. 162-71. Journal code: 8801431. ISSN: 0888-8809. Pub. country: United States. Language: English.

AB Androgen, glucocorticoid, and progesterone receptors (ARs, GRs, and PRs) often can regulate transcription via composite hormone response elements in target genes. We have used artificial and natural mutant ARs from patients with androgen resistance to study their effects on **dominant negative** activity on wild type AR, GR, and PR function on mouse mammary tumor virus (MMTV) and tyrosine aminotransferase (**TAT**) promoters. Artificial ARs that contained internal deletions within the amino-terminal region had minimal transcriptional activity but blocked ligand-mediated transcription by wild type AR. Mutants containing deletions of the DNA-binding and ligand-binding domains had minimal or weak **dominant negative** activity. We then tested the ability of wild type and mutant ARs to modulate GR- and PR-mediated transcriptional activity. The amino-terminal deletion mutants exerted **dominant negative** effects on GR- and PR-mediated activity, both in the absence and presence of testosterone. Surprisingly, wild type AR, which had approximately 20% of the maximal transcriptional activity of GR on the MMTV promoter, also had **dominant negative** activity on dexamethasone-regulated transcription mediated by GR. This **dominant negative** activity likely involves DNA binding because a point mutation in the DNA-binding domain abrogated such activity of an amino-terminal deletion mutant. Additionally, natural human AR mutants from patients with androgen resistance, which do not bind either DNA or ligand, did not block dexamethasone-mediated transcription. In summary, these studies suggest that mutant and wild type ARs can display **dominant negative** activity on other steroid hormone receptors that bind to a composite hormone response element This cross-regulation may be important in regulating maximal transcriptional activity in tissues where these receptors are coexpressed and may contribute to the phenotype of patients with steroid hormone resistance.

L28 ANSWER 88 OF 117 MEDLINE on STN

97048089. PubMed ID: 8892930. Inhibition of human immunodeficiency virus type 1 and type 2 **Tat** function by **transdominant Tat** protein localized to both the nucleus and cytoplasm. Orsini M J; Debouck C M. (Department of Molecular Genetics, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania 19406, USA.) Journal of virology, (1996 Nov) Vol. 70, No. 11, pp. 8055-63. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We introduced various mutations into the activation and RNA binding domains of human immunodeficiency virus type 1 (HIV-1) **Tat** in order to develop a novel and potent **transdominant Tat** protein and to characterize its mechanism of action. The different mutant **Tat** proteins were characterized for their abilities to activate the HIV LTR and inhibit the function of wild-type **Tat** in trans. A **Tat** protein containing a deletion of the basic domain (**Tat**(delta)49-57) localized exclusively to the cytoplasm of transfected human cells was nonfunctional and inhibited both HIV-1 and HIV-2 **Tat** function in a **transdominant** manner. **Tat** proteins containing mutations in the cysteine-rich and core domains were nonfunctional but failed to inhibit **Tat** function in trans. When **Tat** nuclear or nucleolar localization signals were fused to the carboxy terminus of **Tat**(delta)49-57, the chimeric proteins localized to the nucleus or nucleolus, respectively, and remained capable of acting in a **transdominant** manner. Introduction of secondary mutations in the cysteine-rich and core domains of the various **transdominant Tat** proteins completely eliminated their abilities to act in a **transdominant** fashion. Our data best support a mechanism in which these **transdominant Tat** proteins squelch a cellular factor or factors that interact with the **Tat** activation domain and are required for **Tat** to function.

L28 ANSWER 89 OF 117 MEDLINE on STN

96323087. PubMed ID: 8709193. **Transdominant** mutants of I kappa B alpha block **Tat**-tumor necrosis factor synergistic activation of human immunodeficiency virus type 1 gene expression and virus multiplication. Beauparlant P; Kwon H; Clarke M; Lin R; Sonenberg N; Wainberg M; Hiscott J. (Lady Davis Institute for Medical Research, Montreal, Quebec, Canada.) Journal of virology, (1996 Sep) Vol. 70, No. 9, pp. 5777-85. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) contains two binding sites for the NF-kappa B/Rel family of transcription factors which are required for the transcriptional activation of viral genes by inflammatory cytokines such as tumor necrosis factor alpha (TNF-alpha) and interleukin-1. In the present study, we examined the effect of **transdominant** mutants of I kappa B alpha on the synergistic

Tat, in Jurkat T cells. The synergistic induction of HIV-1 LTR-driven gene expression represented a 50- to 70-fold stimulation and required both an intact HIV-1 enhancer and **Tat**-TAR element interaction, since mutations in **Tat** protein (R52Q, R53Q) or in the bulge region of the TAR element that eliminated **Tat** binding to TAR were unable to stimulate LTR expression. Coexpression of I kappa B alpha inhibited **Tat**-TNF-alpha activation of HIV LTR in a dose-dependent manner. **Transdominant** forms of I kappa B alpha, mutated in critical serine or threonine residues required for inducer-mediated (S32A, S36A) and/or constitutive (S283A, T291A, T299A) phosphorylation of I kappa B alpha were tested for their capacity to block HIV-1 LTR transactivation. I kappa B alpha molecules mutated in the N-terminal sites were not degraded following inducer-mediated stimulation (t1/2, > 4 h) and were able to efficiently block HIV-1 LTR transactivation. Strikingly, the I kappa B alpha (S32A, S36A) **transdominant** mutant was at least five times as effective as wild-type I kappa B alpha in inhibiting synergistic induction of the HIV-1 LTR. This mutant also effectively inhibited HIV-1 multiplication in a single-cycle infection model in Cos-1 cells, as measured by Northern (RNA) blot analysis of viral mRNA species and viral protein production. These experiments suggest a strategy that may contribute to inhibition of HIV-1 gene expression by interfering with the NF-kappa B/Rel signaling pathway.

L28 ANSWER 90 OF 117 MEDLINE on STN

96256811. PubMed ID: 8676525. Inhibition of human immunodeficiency virus type 1 replication is enhanced by a combination of **transdominant Tat** and Rev proteins. Ulich C; Harrich D; Estes P; Gaynor R B. (Division of Molecular Virology, Department of Medicine, University of Texas Southwestern Medical Center at Dallas, Texas 75235-8594, USA.) Journal of virology, (1996 Jul) Vol. 70, No. 7, pp. 4871-6. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Mutation of either of two critical human immunodeficiency virus type 1 (HIV-1) regulatory proteins, **Tat** and Rev, results in marked defects in viral replication. Thus, inhibition of the function of one or both of these proteins can significantly inhibit viral growth. In the present study, we constructed a novel **transdominant Tat** mutant protein and compared its efficiency in inhibiting HIV-1 replication with that of **transdominant** mutant Rev M10 when these proteins were stably expressed either alone or in combination in T-lymphocyte cell lines. The **transdominant Tat** mutant protein alone resulted in a modest inhibition of HIV replication, but it was able to enhance the ability of the M10 Rev mutant protein to inhibit HIV-1 replication. These results suggest a possible synergistic effect of these **transdominant** mutant proteins in inhibiting HIV-1 replication.

L28 ANSWER 91 OF 117 MEDLINE on STN

96252195. PubMed ID: 8646555. Inhibition of HIV-1 replication and reactivation from latency by **tat transdominant negative** mutants in the cysteine rich region. Caputo A; Grossi M P; Bozzini R; Rossi C; Betti M; Marconi P C; Barbanti-Brodano G; Balboni P G. (Institute of Microbiology, School of Medicine, University of Ferrara, Italy.) Gene therapy, (1996 Mar) Vol. 3, No. 3, pp. 235-45. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **Tat** mutants (tat22, tat37 and tat22/37) were constructed in the transactivation domain, where cysteines at positions 22 or/and 37 were substituted with glycine and serine, respectively. These mutants were expressed either in a BK virus episomal vector or in the retroviral vector LXSN. Constitutive production of tat22 by Jurkat T cells in the context of both vectors blocked HIV-1 replication during lytic infection. Conversely, the tat37 mutant did not show any inhibitory activity and tat22/37 displayed a mild effect on HIV-1 infection only when expressed by the recombinant retrovirus. However, constitutive production of tat22/37 by the BK virus vector in Jurkat T cells chronically infected by HIV-1 was effective in blocking reactivation of viral replication induced by tumor necrosis factor-alpha or human herpesvirus-6. These results suggest that mutants in the transactivation domain of **tat** may be considered in designing alternative strategies to control HIV-1 replication and reactivation from latency during different phases of infection.

L28 ANSWER 92 OF 117 MEDLINE on STN

96238293. PubMed ID: 8787346. [Gene therapy for hereditary and acquired human diseases]. Therapie genique de maladies humaines hereditaires et acquises. Mehtali M; Imler J L; Sorg T; Pavirani A. (TRANSGENE S.A., Strasbourg.) Annales d'endocrinologie, (1995) Vol. 56, No. 6, pp. 571-4. Ref: 15. Journal code: 0116744. ISSN: 0003-4266. Pub. country: France. Language: French.

AB Cystic Fibrosis (CF) and AIDS are primary candidate disorders to be treated by gene therapy, owing to their lethality and the absence of efficient clinical treatments. Treatment of CF by gene therapy will require the transfer of the functional CFTR cDNA into the diseased human airway epithelia since mutations within the CFTR gene are responsible for CF. We have therefore cloned the human CFTR cDNA and developed a recombinant E1-deleted adenoviral vector carrying a CFTR expression

efficiently transduce human lung cells isolated from CF patients and to correct their phenotype. Efficient in, vivo delivery of the CFTR cDNA to the airways of cotton rats and rhesus monkeys was also obtained and no dissemination of the recombinant viral vector in other tissues than the airways was observed. We have therefore designed a phase I clinical trial involving CF patients. In contrast to the monogenic CF disease, the mechanisms of AIDS pathogenesis still remain poorly understood. Such limited knowledge of the disease constitutes a serious restriction to the development of a rational gene therapy strategy for AIDS. Since HIV, the causative agent of AIDS, predominantly infects cells of the hematopoietic system, pluri- or multipotent stem cells may constitute potential targets for the introduction of a foreign anti-HIV gene that will inhibit HIV replication and/or spread. Reimplantation of the genetically modified stem cells into asymptomatic HIV-infected patients should theoretically allow the repopulation of the host's immune system with mature CD4+ cells expressing novel molecules that interfere with viral replication, thus slowing the progression of AIDS. We identified several new **transdominant** inhibitors derived from the viral **TAT** and REV proteins and showed their ability to confer to human CD4 lymphocytes resistance against HIV1 infection. Retroviral vectors carrying these potential therapeutic genes have been developed and are currently being tested in vivo in newly developed transgenic animal models, in humanized SCID mice and in macaques.

L28 ANSWER 93 OF 117 MEDLINE on STN
96144820. PubMed ID: 8551047. Detection of intracellular HIV-1 Rev protein by flow cytometry. Rigg R J; Dando J S; Escaich S; Plavec I; Bohnlein E. (Progenesys, Palo Alto, CA 94304, USA.) Journal of immunological methods, (1995 Dec 27) Vol. 188, No. 2, pp. 187-95. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB The Rev trans-activator protein plays a pivotal role in human immunodeficiency virus type 1 (HIV-1) replication by allowing expression of the viral structural proteins. We have developed a protocol to quantitatively assay intracellular steady state levels of Rev Ag (Rev wild type and RevM10 proteins) by flow cytometry. Three fixation and permeabilization techniques were compared. These protocols varied in the magnitude of the signal which could be detected, and in the ability to distinguish between Rev Ag positive and negative populations. This technology is applicable to a variety transduced or transfected cell types (species, lineage), and for cell lines and primary cells acutely infected with HIV-1. The assay is therefore a valuable tool both to analyze Rev protein expression levels in HIV-infected cells and to optimize delivery of the **dominant-negative** RevM10 gene for clinical gene therapy applications. In addition, a second, independent intracellular protein (HIV-**Tat**) has been detected using the same approach.

L28 ANSWER 94 OF 117 MEDLINE on STN
96050917. PubMed ID: 7584057. Regulated expression of a **dominant negative** form of Rev improves resistance to HIV replication in T cells. Liu J; Woffendin C; Yang Z Y; Nabel G J. (Howard Hughes Medical Institute, University of Michigan Medical Center, Department of Internal Medicine, Ann Arbor 48109-0650, USA.) Gene therapy, (1994 Jan) Vol. 1, No. 1, pp. 32-7. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Infection by the human immunodeficiency virus (HIV) has remained refractory to treatment, and molecular genetic interventions have been developed for the treatment of the acquired immunodeficiency syndrome (AIDS). Previous studies have focused on the development of gene products which inhibit productive HIV replication, including **transdominant** proteins, RNA decoys and ribozymes. In this report, we show that appropriate expression vectors which optimize production and regulated synthesis of a **transdominant** mutant form of Rev improve its antiviral effect. The combination of a strong constitutive enhancer, a **Tat** activation response (TAR) regulatory element and **transdominant** Rev take advantage of three aspects of early viral gene expression to confer increased resistance to HIV replication. This vector may be useful, alone or in combination with other antiviral genes, to provide gene therapy for AIDS.

L28 ANSWER 95 OF 117 MEDLINE on STN
96050916. PubMed ID: 7584056. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 **tat** and gag. Lori F; Lisziewicz J; Smythe J; Cara A; Bunnag T A; Curiel D; Gallo R C. (Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, USA.) Gene therapy, (1994 Jan) Vol. 1, No. 1, pp. 27-31. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Efficient transduction of inhibitory genes is a critical requirement in the development of a gene therapy strategy against human immunodeficiency virus type 1 (HIV-1). Commonly used systems based on retrovirus-mediated gene delivery are characterized by low efficiency gene transfer into the

60-90% of human CD4+ cells by using a novel technique that allows high efficiency gene transfer mediated by adenoviruses coupled with DNA-polylysine complexes. Protection of these cells against HIV-1 acute infection was evaluated by transducing them with three different inhibitory genes which interfere with HIV-1 replication at separate levels (polymeric **Tat** activation response element [TAR] decoy, **dominant-negative** mutant of the gag gene and antisense sequences of the gag gene) and subsequent challenging with HIV-1. The polymeric TAR decoy inhibited HIV-1 replication over 95%. Both the **dominant-negative** mutant and the antisense sequence of the gag gene were less potent inhibitors than the polymeric-TAR decoy. Combinations of either polymeric-TAR with **dominant-negative** mutant or antisense of the gag gene synergistically enhanced the inhibitory effects of the single genes. These data suggest that the combination of a highly efficient transduction technique with effective HIV-1 inhibitory genes confers rapid protection against HIV-1 acute infection in vitro.

L28 ANSWER 96 OF 117 MEDLINE on STN
96022225. PubMed ID: 7475320. Gene therapy against retroviral diseases. Essex M; Matsuda Z; Yu X; Lee T H. (Department of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts 02115, USA.) Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K, (1995 Oct) Vol. 9 Suppl 1, pp. S71-4. Ref: 13. Journal code: 8704895. ISSN: 0887-6924. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Eventually, gene therapy may be a valid option for chronic viral infections, including retroviral infections. Human retroviral diseases fit two categories: (1) those that result from a monoclonal outgrowth of a human T-cell leukemia virus type I (HTLV-I)-infected cell, as in the case of adult T cell leukemia (ATL); and (2) those that appear to result directly from virus load rather than monoclonal outgrowth--such as tropical spastic paraparesis/HTLV-I associated myelopathy (TSP/HAM) and human immunodeficiency virus (HIV)-associated acquired immune deficiency syndrome (AIDS). For ATL gene therapy, corrective mechanisms directed at regulatory sequences rather than viral sequences may be most important, though perhaps anti-tax therapy would be useful. For TSP/HAM and AIDS, gene therapy directed to control virus replication may be most useful. For anti-retroviral therapy, one may use **dominant negative** mutants and a variety of other approaches that direct toxins or compete out viral regulatory gene signal sequences. For maximum benefit, such therapy should be directed to different essential genes (eg gag, pol, env, **tat** or rev) involved in the virus replication cycle and utilize different toxic approaches. A major impediment to the use of gene therapy for AIDS is our inability to transfect a significant fraction of target cells in vivo. Except for reconstituted mice, retroviral systems of animals have been under-utilized as models for gene therapy. Naturally occurring retroviral diseases of cats, goats, horses, and other species provide models for future development.

L28 ANSWER 97 OF 117 MEDLINE on STN
95396000. PubMed ID: 7666562. Transcriptional silencing of human immunodeficiency virus type 1 long terminal repeat-driven gene expression by the Kruppel-associated box repressor domain targeted to the transactivating response element. Pengue G; Caputo A; Rossi C; Barbanti-Brodano G; Lania L. (Department of Genetics, Molecular and General Biology, University of Naples, Federico II, Italy.) Journal of virology, (1995 Oct) Vol. 69, No. 10, pp. 6577-80. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The evolutionarily conserved protein domain, called the Kruppel-associated box (KRAB), present in the amino termini of a large number of Kruppel-type zinc finger proteins is a strong repressor domain. In order to develop novel strategies to control human immunodeficiency virus type 1 (HIV-1) gene expression, we constructed a series of expression vectors expressing the wild-type **Tat** or **Tat transdominant negative** mutants fused to the KRAB repressor domain. We found that the KRAB domain tethered to the transactivating response element is able to suppress both basal and **Tat**-mediated activity of HIV-1 long terminal repeat-driven gene expression. These results suggest that the KRAB repressor domain fused to the **Tat transdominant negative** mutants can be successfully employed to control HIV-1 gene expression.

L28 ANSWER 98 OF 117 MEDLINE on STN
95339003. PubMed ID: 7614248. Inhibition of HIV-1 by a double **transdominant** fusion gene. Aguilar-Cordova E; Chinen J; Donehower L A; Harper J W; Rice A P; Butel J S; Belmont J W. (Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030, USA.) Gene therapy, (1995 May) Vol. 2, No. 3, pp. 181-6. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A double **transdominant** fusion gene (trev) designed to inhibit two essential HIV functions simultaneously was constructed by linking **tat** and rev **transdominant** mutants. Trev independently inhibited both **Tat** and Rev functions, localized within the nucleus and cells transfected with

retroviral vector of trev was made and shown also to confer protection from HIV cytopathic effects. Simultaneous inhibition of two essential viral genes presents significant advantages for potential gene therapy treatment of HIV infection over conventional single effect molecules.

L28 ANSWER 99 OF 117 MEDLINE on STN

95287453. PubMed ID: 7769662. Inhibition of clinical human immunodeficiency virus (HIV) type 1 isolates in primary CD4+ T lymphocytes by retroviral vectors expressing anti-HIV genes. Vandendriessche T; Chuah M K; Chiang L; Chang H K; Ensoli B; Morgan R A. (Clinical Gene Therapy Branch, National Cancer Institute, Bethesda, Maryland 20892, USA.) Journal of virology, (1995 Jul) Vol. 69, No. 7, pp. 4045-52. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Gene therapy may be of benefit in human immunodeficiency virus type 1 (HIV-1)-infected individuals by virtue of its ability to inhibit virus replication and prevent viral gene expression. It is not known whether anti-HIV-1 gene therapy strategies based on antisense or **transdominant** HIV-1 mutant proteins can inhibit the replication and expression of clinical HIV-1 isolates in primary CD4+ T lymphocytes. We therefore transduced CD4+ T lymphocytes from uninfected individuals with retroviral vectors expressing either HIV-1-specific antisense-TAR or antisense-**Tat**/Rev RNA, **transdominant** HIV-1 Rev protein, and a combination of antisense-TAR and **transdominant** Rev. The engineered CD4+ T lymphocytes were then infected with four different clinical HIV-1 isolates. We found that replication of all HIV-1 isolates was inhibited by all the anti-HIV vectors tested. Greater inhibition of HIV-1 was observed with **transdominant** Rev than with antisense RNA. We hereby demonstrated effective protection by antisense RNA or **transdominant** mutant proteins against HIV-1 infection in primary CD4+ T lymphocytes using clinical HIV-1 isolates, and this represents an essential step toward clinical anti-HIV-1 gene therapy.

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E HARPER J WADE/IN
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L5 0 S L4 NOT L1

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FILE 'MEDLINE' ENTERED AT 08:28:23 ON 27 JUN 2006
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L11 77 S E2-E6
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L27         7253 S TAT
L28          117 S L27 AND (TRANSDOMINANT OR TRANS-DOMINANT OR DOMINANT NEGATIVE

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       1397862 HUMAN
        123524 IMMUNODEFICIENCY
        415203 VIRUS
         48706 HUMAN IMMUNODEFICIENCY VIRUS
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L30          2 L29 AND TREV

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L30  ANSWER 1 OF 2     MEDLINE on STN
97288989.    PubMed ID: 9143912.   Protection of primary human T cells from
     HIV infection by Trev: a transdominant fusion gene. Chinen J;
     Aguilar-Cordova E; Ng-Tang D; Lewis D E; Belmont J W. (Department of
     Microbiology and Immunology, Baylor College of Medicine, Houston, TX
     77030, USA. ) Human gene therapy, (1997 May 1) Vol. 8, No. 7, pp. 861-8.
     Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States.
     Language: English.

L30  ANSWER 2 OF 2     MEDLINE on STN
95339003.    PubMed ID: 7614248.   Inhibition of HIV-1 by a double
     transdominant fusion gene. Aguilar-Cordova E; Chinen J; Donehower L A;
     Harper J W; Rice A P; Butel J S; Belmont J W. (Department of Pediatrics,
     Baylor College of Medicine, Houston, TX 77030, USA. ) Gene therapy, (1995
     May) Vol. 2, No. 3, pp. 181-6.  Journal code: 9421525. ISSN: 0969-7128.
     Pub. country: ENGLAND: United Kingdom. Language: English.


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     FILE 'MEDLINE' ENTERED AT 08:28:23 ON 27 JUN 2006
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L27         7253 S TAT
L28          117 S L27 AND (TRANSDOMINANT OR TRANS-DOMINANT OR DOMINANT NEGATIVE
L29       165361 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L30            2 S L29 AND TREV

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E4            5     CORDOVA COLLEEN W/IN
E5            1     CORDOVA DANIEL/IN
E6           14     CORDOVA DAVID S/IN
E7            4     CORDOVA DAVID SOLARES/IN
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E9              1      CORDOVA ELMER/IN
E10             1      CORDOVA ELMER R/IN
E11             1      CORDOVA FRANK L/IN
E12             1      CORDOVA GARY A/IN

=> e aguilar cordova c e/in
E1              2      AGUILAR CARLOS L/IN
E2              1      AGUILAR CARMEN ARNALOT I/IN
E3              0 --> AGUILAR CORDOVA C E/IN
E4              1      AGUILAR CORDOVA C ESTUARDO/IN
E5              1      AGUILAR CORDOVA CARLOS ESTUARDO/IN
E6              5      AGUILAR CORDOVA ESTUARDO/IN
E7              1      AGUILAR CORDOVA ESTUARDO C/IN
E8              2      AGUILAR CORINNE/IN
E9              6      AGUILAR DANIEL A/IN
E10             1      AGUILAR DANIEL ALFONSO/IN
E11             2      AGUILAR DAVID G/IN
E12             1      AGUILAR DENNIS/IN

=> s e4-e7
                1 "AGUILAR CORDOVA C ESTUARDO"/IN
                1 "AGUILAR CORDOVA CARLOS ESTUARDO"/IN
                5 "AGUILAR CORDOVA ESTUARDO"/IN
                1 "AGUILAR CORDOVA ESTUARDO C"/IN
L1              8 ("AGUILAR CORDOVA C ESTUARDO"/IN OR "AGUILAR CORDOVA CARLOS
                  ESTUARDO"/IN OR "AGUILAR CORDOVA ESTUARDO"/IN OR "AGUILAR CORDOV
                 A ESTUARDO C"/IN)

=> d l1,ti,1-8

L1   ANSWER 1 OF 8  USPATFULL on STN
TI     Chimeric viral vectors for gene therapy

L1   ANSWER 2 OF 8  USPATFULL on STN
TI     Double transdominant fusion gene and protein

L1   ANSWER 3 OF 8  USPATFULL on STN
TI     Recombinant adenoviral vectors and their utility in the treatment of
       various types of fibrosis: hepatic, renal, pulmonary, as well as
       hypertrophic scars

L1   ANSWER 4 OF 8  USPATFULL on STN
TI     Recombinant adenoviral vectors and their utility in the treatment of
       various types of fibrosis: hepatic, renal, pulmonary, as well as
       hypertrophic scars

L1   ANSWER 5 OF 8  USPATFULL on STN
TI     Recombinant viral and non-viral vectors containing the human urokinase
       plasminogen activator gene and its utilization in the treatment of
       various types of hepatic, pulmonary, pancreatic and cardiac fibrosis and
       hypertrophic scars

L1   ANSWER 6 OF 8  USPATFULL on STN
TI     Chemeric viral vectors for gene therapy

L1   ANSWER 7 OF 8  USPATFULL on STN
TI     Aldehyde reductase bidirectional promoter and its use

L1   ANSWER 8 OF 8  USPATFULL on STN
TI     Recombinant adenoviral vectors and their utility in the treatment of
       various types of fibrosis: hepatic, renal, pulmonary, as well as
       hypertrophic scars


=> d l1,cbib,clm,1-8

L1   ANSWER 1 OF 8  USPATFULL on STN
2006:67334 Chimeric viral vectors for gene therapy.
    Aguilar-Cordova, Estuardo, Boston, MA, UNITED STATES
    US 2006057553 A1 20060316
    APPLICATION: US 2001-297341  A1  20010530 (10)
    WO 2001-US17453 20010530 20030523  PCT 371 date
    PRIORITY: US 2000-207845P  20000530 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM    What is claimed is:
       1. A chimeric nucleic acid vector comprising:  (a) adenoviral inverted
       terminal repeat flanking regions;  (b) an internal region between said
       adenoviral flanking regions, wherein said internal region contains
       retroviral long terminal repeat flanking regions flanking a cassette,
       wherein said cassette contains a nucleic acid region of interest; and
       (c) a gag nucleic acid region between said adenoviral flanking regions.
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2. A chimeric nucleic acid vector comprising: (a) adenoviral inverted terminal repeat flanking regions; (b) an internal region between said adenoviral flanking regions, wherein said internal region contains retroviral long terminal repeat flanking regions flanking a cassette, wherein said cassette contains a nucleic acid region of interest; and (d) a pol nucleic acid region between said adenoviral flanking regions.

3. A chimeric nucleic acid vector comprising: (a) adenoviral inverted terminal repeat flanking regions; (b) an internal region between said adenoviral flanking regions, wherein said internal region contains retroviral long terminal repeat flanking regions flanking a cassette, wherein said cassette contains a nucleic acid region of interest; and (c) a nucleic acid region between said adenoviral flanking regions selected from the group consisting of an env nucleic acid region, a nucleic acid region for pseudotyping a retroviral vector and a nucleic acid region for targeting a retroviral vector.

4. A chimeric nucleic acid vector comprising: (a) adenoviral inverted terminal repeat flanking regions; (b) an internal region between said adenoviral flanking regions, wherein said internal region contains retroviral long terminal repeat flanking regions flanking a cassette, wherein said cassette contains a nucleic acid region of interest; (c) a gag nucleic acid region between said adenoviral flanking regions; and (e) a pol nucleic acid sequence between said adenoviral flanking regions.

5. A chimeric nucleic acid vector comprising: (a) adenoviral inverted terminal repeat flanking regions; (b) an internal region between said adenoviral flanking regions, wherein said internal region contains retroviral long terminal repeat flanking regions flanking a cassette, wherein said cassette contains a nucleic acid region of interest; (c) a gag nucleic acid region between said adenoviral flanking regions; (f) a pol nucleic acid region between said adenoviral flanking regions; and (g) a nucleic acid region between said adenoviral flanking regions selected from the group consisting of an env nucleic acid region, a nucleic acid region for pseudotyping a retroviral vector and a nucleic acid region for targeting a retroviral vector.

6. A chimeric nucleic acid vector comprising: (a) adenoviral inverted terminal repeat flanking regions; (b) an internal region between said adenoviral flanking regions, wherein said internal region contains retroviral long terminal repeat flanking regions flanking a cassette, wherein said cassette contains a nucleic acid region of interest; (c) a gag nucleic acid region between said adenoviral flanking regions; (d) a pol nucleic acid region between said adenoviral flanking regions; (d) a nucleic acid region between said adenoviral flanking regions selected from the group consisting of an env nucleic acid region, a nucleic acid region for pseudotyping a retroviral vector and a nucleic acid region for targeting a retroviral vector; and (e) a suicide nucleic acid region between said adenoviral flanking regions.

7. The chimeric nucleic acid vector of claims 3, 5, or 6, wherein a transactivator nucleic acid region is located between said adenoviral flanldng regions, and wherein said transactivator nucleic acid region encodes a polypeptide which regulates expression of said env nucleic acid.

8. The vector of claim 7, wherein said transactivator is the tetracycline transactivator.

9. The chimeric nucleic acid vector of claim 3, 5 and 6, wherein the expression of said env nucleic acid region is regulated by an inducible promoter nucleic acid region.

10. The chimeric nucleic acid vector of claim 9, wherein said inducible promoter nucleic acid region is induced by a stimulus selected from the group consisting of tetracycline, galactose, glucocorticoid, Ru487, and heat shock.

11. The chimeric nucleic acid vector of claim 3, 5 or 6 wherein said env nucleic acid region is selected from the group consisting of amphotropic envelope, xenotropic envelope, ecotropic envelope, human immunodeficiency virus 1 (HIV-1) envelope, human immunodeficiency virus 2 (HIV-2) envelope, feline immunodeficiency virus (FIV) envelope, simian immunodeficiency virus 1(SIV) envelope, human T-cell leukemia virus 1 (HTLV-1) envelope, human T-cell leukemia virus 2 (HTLV-2) envelope and vesicular stomatis virus-G glycoprotein.

12. The chimeric nucleic acid vector of claim 6 wherein said suicide nucleic acid region is selected from the group consisting of Herpes simplex virus type 1 thymidise kinase, oxidoreductase, cytosine deaminase, thymidine kinase thymidilate idnase (Tdk::Tmk) and

13. A chimeric plasmid comprising: (a) adenoviral inverted terminal repeat flanking regions; (b) an internal region between said adenoviral flanking regions, wherein said internal region contains retroviral long terminal repeat flanking regions flanking a cassette, wherein said cassette contains a nucleic acid region of interest; (c) a gag nucleic acid region; (d) a pol nucleic acid region; and (e) a nucleic acid region between said adenoviral flanking regions selected from the group consisting of an env nucleic acid region, a nucleic acid region for pseudotyping a retroviral vector and a nucleic acid region for targeting a retroviral vector.

14. The chimeric nucleic acid plasmid of claim 11, further comprising a suicide nucleic acid.

15. The chimeric nucleic acid plasmid of claim 11, wherein plasmid further comprises a transactivator nucleic acid region, wherein said transactivator nucleic acid region encodes a polypeptide which regulates expression of said env nucleic acid region.

16. A chimeric nucleic acid vector comprising: (a) adenoviral inverted terminal repeat flanking regions; (b) an internal region between said adenoviral flanking regions, wherein said internal region contains adeno-associated viral inverted terminal repeat flanking regions flanking a cassette, wherein said cassette contains a nucleic acid region of interest; and (b) a rep nucleic acid region between said adenoviral flanking regions.

17. A chimeric nucleic acid vector comprising: (a) adenoviral inverted terminal repeat flanking regions; (b) an internal region between said adenoviral flanking regions, wherein said internal region contains adeno-associated viral inverted terminal repeat flanking regions flanking a cassette, wherein said cassette contains a nucleic acid region of interest; and (b) a cap nucleic acid region between said adenoviral flanking regions.

18. A chimeric nucleic acid vector comprising: (a) adenoviral inverted terminal repeat flanking regions; (b) an internal region between said adenoviral flanking regions, wherein said internal region contains adeno-associated viral inverted terminal repeat flanking regions flanking a cassette, wherein said cassette contains a nucleic acid region of interest; and (b) an adenoviral E4 nucleic acid region between said adenoviral flanking regions.

19. A chimeric nucleic acid vector comprising: (a) adeno-associated viral inverted terminal repeat flanking regions; (b) an internal region between said adeno-associated viral flanking regions, wherein said internal region contains retroviral long terminal repeat flanking regions flanking a cassette, wherein said cassette contains a nucleic acid region of interest; (c) a rep nucleic acid region between said adenoviral flanking regions; (d) a cap nucleic acid region between said adenoviral flanking regions; and (e) an adenoviral E4 nucleic acid region between said adenoviral flanking regions.

20. A method for producing retroviral virions comprising: a) producing a chimeric nucleic acid vector comprising: (i) adenoviral inverted terminal repeat flanking regions; (ii) an internal region between said adenoviral flanking regions, wherein said internal region contains retroviral long terminal repeat flanking regions flanking a cassette, wherein said cassette contains a nucleic acid region of interest; b) introducing said chimeric nucleic acid vector to a cell, wherein said cell comprises a gag nucleic acid region, a pol nucleic acid region, an env nucleic acid region and a replication-defective helper vector, wherein said helper vector comprises E1 and E3 nucleic acid region; and c) producing an infectious retroviral virion.

21. A method for producing retroviral virions comprising: a) producing a chimeric nucleic acid vector comprising: (i) adenoviral inverted terminal repeat flanking regions; (ii) an internal region between said adenoviral flanking regions, wherein said internal region contains retroviral long terminal repeat flanking regions flanking a cassette, wherein said cassette contains a nucleic acid region of interest; b) introducing said chimeric nucleic acid vector to a cell, wherein said cell comprises a gag nucleic acid region, a pol nucleic acid region and an env nucleic acid region; c) introducing to said cell a replication-defective helper vector, wherein said helper vector comprises E1 and E3 nucleic acid region; and d) producing an infectious retroviral virion.

22. The method of claim 20 or 21 wherein both of said introducing steps occur concomitantly.

selected from the group consisting of amphotropic envelope, xenotropic envelope, ecotropic envelope, human immunodeficiency virus 1 (HIV-1) envelope, human immunodeficiency virus 2 (HIV-2) envelope, feline immunodeficiency virus (FIV) envelope, simian immunodeficiency virus 1 (SIV) envelope, human T-cell leukemia virus 1 (HTLV-1) envelope, human T-cell leukemia virus 2 (HTLV-2) envelope and vesicular stomatis virus-G glycoprotein.

24. A method for producing retroviral virions comprising: a) producing a chimeric nucleic acid vector comprising: (i) adenoviral inverted terminal repeat flanking regions; (ii) an internal region between said adenoviral flanking regions, wherein said internal region contains retroviral long terminal repeat flang regions flanking a cassette, wherein said cassette contains a nucleic acid region of interest; (iii) a gag nucleic acid region between said adenoviral flanking regions; (iv) apol nucleic acid region between said adenoviral flanking regions; and (v) a nucleic acid region between said adenoviral flanking regions selected from the group consisting of an env nucleic acid region, a nucleic acid region for pseudotyping a retroviral vector and a nucleic acid region targeting a retroviral vector; b) introducing said chimeric nucleic acid vector to a cell, wherein said cell comprises a replication-defective helper vector, wherein said helper vector comprises E1 and E3 nucleic acid region; and c) producing an infectious retroviral virion.

25. A method for producing retroviral virions comprising: a) producing a chimeric nucleic acid vector comprising: (i) adenoviral inverted terminal repeat flanking regions; (ii) an internal region between said adenoviral flanking regions, wherein said internal region contains retroviral long terminal repeat flanking regions flanking a cassette, wherein said cassette contains a nucleic acid region of interest; (iii) a gag nucleic acid region between said adenoviral inverted terminal repeat flanking regions; (iv) a pol nucleic acid region between said adenoviral inverted terminal repeat flanking regions; and (iv) a nucleic acid region between said adenoviral flanking regions selected from the group consisting of an env nucleic acid region, a nucleic acid region for pseudotyping a retroviral vector and a nucleic acid region targeting a retroviral vector; and b) introducing said chimeric nucleic acid vector to a cell; c) introducing to said cell a replication-defective helper vector, wherein said helper vector comprises E1 and E3 nucleic acid region; and d) producing an infectious retroviral virion.

26. The method of claim 24 or 25 further comprising transduction of said infectious retroviral virion to another cell.

27. The method of claim 26 wherein said another cell is a hepatocyte.

28. The method of claim 20, 21, 24 or 25, wherein said cell further comprises a packaging region.

29. The chimeric nucleic acid vector of claim 1, 2, 3, 4, 5, 6, 11, 14, 15, 16, or 17, wherein said nucleic acid region of interest is selected from the group consisting of a reporter region, ras, myc, raf; erb, src, fms, jun, trk, ret, gsp, hst, bcl abl, Rb, CFTR, p16, p21, p27, p53, p57, p73, C-CAM, APC, CTS-1, zacl, scFV ras, DCC, NF-1, NF-2, WT-1, MFN-I, MEN-II, BRCA1, VHL, MMAC1, FCC, MCC, BRCA2, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11 IL-12, GM-CSF G-CSF, thymidine kinase, CD40L, Factor VIII, Factor IX, CD40, multiple disease resistance (MDR), ornithine transcarbamylase (OTC), ICAM-1, and insulin receptor.

30. The method of claim 20, 21, 24 or 25, wherein said nucleic acid region of interest is selected from the group consisting of a reporter region, ras, myc, raf, erb, src, fms, jun, trk, ret, gsp, hst, bcl abl, Rb, CFTR, p16, p21, p27, p53, p57, p73, C-CAM, APC, CTS-1, zacl, scFV ras, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, BRCA1, VHL, MMAC1, FCC, MCC, BRCA2, IL-1, IL-2, 1IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11 IL-12, GM-CSF G-CSF, thymidine kinase, CD40L, Factor VIII, Factor IX, CD40, multiple disease resistance (MDR), ornithine transcarbamylase (OTC), ICAM-1, and insulin receptor.

L1 ANSWER 2 OF 8 USPATFULL on STN
2005:299052 Double transdominant fusion gene and protein.
Aguilar-Cordova, C. Estuardo, Houston, TX, UNITED STATES
Belmont, John W., Houston, TX, UNITED STATES
Harper, J. Wade, Sugar Land, TX, UNITED STATES
Rice, Andrew, Houston, TX, UNITED STATES
Butel, Janet S., Houston, TX, UNITED STATES
Chinen, Javier, Houston, TX, UNITED STATES
US 2005260717 A1 20051124

DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. (canceled)

2. (canceled)

3. (canceled)

4. (canceled)

5. (canceled)

6. An isolated double transdominant fusion gene, comprising: a tat transdominant mutant gene, wherein codons of said tat mutant gene which code for basic amino acids at positions 52 to 57 of the Tat protein are replaced with codons which code for neutral amino acids; a rev transdominant mutant gene, wherein the codons of the rev mutant which code for amino acids at positions 80 to 82 of the Rev protein have been deleted; and codon coding for histidine, wherein said histidine comprises a histidine bridge that links the Tat transdominant mutant protein to the Rev transdominant mutant protein.

7. (canceled)

8. (canceled)

9. A vector comprising said isolated double transdominant fusion gene of claim 6, wherein said gene is operably linked to a promoter.

10. A transdominant protein produced by the vector of claim 9.

11. An isolated double transdominant fusion gene, comprising a tat transdominant mutant gene having a substituted basic domain in which basic amino acids are replaced by neutral amino acids, said tat transdominant mutant gene is operably linked to a rev transdominant mutant gene having a deletion in a leucine-rich coding domain, wherein the double transdominant fusion gene product inhibits HIV expression.

12. The transdominant fusion gene of claim 11, wherein the tat and rev transdominant mutant genes are linked by a codon which codes for a histidine bridge.

13. A vector comprising said isolated double transdominant fusion gene of claim 11, wherein said gene is operably linked to a promoter.

14. A transdominant protein produced by the vector of claim 13.

15. A method of inhibiting HIV replication in a HIV infected cell in vitro, comprising: delivering to the HIV infected cell in vitro an effective amount of the vector of claim 13, from which is expressed the double transdominant fusion gene product.

L1 ANSWER 3 OF 8 USPATFULL on STN
2005:233050 Recombinant adenoviral vectors and their utility in the treatment of various types of fibrosis: hepatic, renal, pulmonary, as well as hypertrophic scars.
Armendariz Borunda, Juan, Prado Coapa, MEXICO
Aguilar Cordova, Estuardo, Col. Prado Coapa, MEXICO
TGT Laboratories, S.A. DE C.V. (non-U.S. corporation)
US 2005201984 A1 20050915
APPLICATION: US 2005-64504 A1 20050224 (11)
PRIORITY: MX 1999-998515 19990917
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1-21. (canceled)

22. A recombinant adenoviral vector comprising an adenoviral genome replaced with a therapeutic gene or DNA sequence regulated by a ubiquitous promoter represented by a Cytomegalovirus (CMV) promotor or a tissue-specific phosphoenolpyruvate carboxykinase promoter, wherein the therapeutic gene or DNA sequence encodes for a latent human metalloprotease gene MMP-8.

23. The recombinant adenoviral vector of claim 22, wherein the recombinant adenoviral vector is pAdGFP-MMP-8 or paDCMV-MMP-8.

24. The recombinant adenoviral vector of claim 23, wherein the metalloprotease gene MMP-8 can be delivered to an organ by an administrative route.

25. The recombinant adenoviral vector of claim 24, wherein the organ is selected from the group consisting of liver, lung, heart, kidney, skin, hypertrophic scars, and combinations thereof.

26. The recombinant adenoviral vector of claim 25, wherein the organ is the liver.

27. The recombinant adenoviral vector of claim 24, wherein the administrative route is endovenous.

28. A method of preparing a recombinant adenoviral vector comprising cloning a reporter gene and a therapeutic gene or DNA sequence, wherein the therapeutic gene or DNA sequence, wherein the therapeutic gene or DNA sequence is regulated by either a phosphoenolpyruvate carboxykinase promoter or a cytomegalovirus promotor and wherein the therapeutic gene or DNA sequence encodes for a collagenase or metalloprotease.

29. The method of claim 28, wherein the reporter gene are selected from the group consisting of Lac-Z and GFP.

30. The method of claim 28, wherein the metalloprotease is selected from the group consisting of a latent human metalloprotease gene MMP-8 and an active human metalloprotease gene MMP-8.

31. The method of claim 28, wherein the recombinant adenoviral vector is pAdGFP-MP-8.

32. A recombinant adenoviral vector comprising an adenoviral genome containing a therapeutic DNA sequence regulated by a cytomegalovirus promotor, wherein the therapeutic DNA sequence encodes for a collagenase or metalloproteaser.

33. The recombinant adenoviral vector of claim 32, wherein the metalloprotease is selected from the group consisting of a latent human metalloprotease gene MMP-8 and an active human metalloprotease gene MMP-8.

34. A recombinant adenoviral vector that contains an adenoviral genome wherein open reading frames E1, E3, or both E1 and E3 from have been deleted but retain enough sequence to make the adenoviral vector able to replicate in vivo, said vector containing a therapeutic gene or DNA sequence regulated by a ubiquitous promotor or tissue specific promotor that encodes for a therapeutic protein.

35. The recombinant adenoviral vector of claim 34, wherein the tissue specific promotor is PEPCK.

36. The recombinant adenoviral vector of claim 34, wherein the ubiquitous promotor is a cytomegalovirus (CMV) or Rous sarcoma virus (RSV) promotor.

37. The recombinant adenoviral vector of claim 34 comprising a viral vector.

38. The recombinant adenoviral vector of claim 34 comprising a non-viral vector.

39. The recombinant adenoviral vector of claim 38, wherein the non-viral vector is selected from the group consisting of a plasmid, a cationic liposome, and an anionic liposome.

40. A process for manufacturing the recombinant adenoviral vector of claim 34, comprising cloning reporter genes La-Z and GFP and the therapeutic gene or DNA sequence.

41. A pharmaceutical composition comprising the recombinant adenoviral vector of claim 34 and a pharmaceutically acceptable carrier.

42. The pharmaceutical composition of claim 41, wherein a unitary dose comprises between 10^7 and 10^{14} viral particles.

L1 ANSWER 4 OF 8 USPATFULL on STN
2004:202938 Recombinant adenoviral vectors and their utility in the treatment of various types of fibrosis: hepatic, renal, pulmonary, as well as hypertrophic scars.
Armendariz Borunda, Juan, Col. Prado Coapa, MEXICO
Aguilar Cordova, Estuardo, Col. Prado Coapa, MEXICO
TGT Laboratories, S.A. DEC. V, Col. Prado Coapo, MEXICO (non-U.S. corporation)
US 2004156827 A1 20040812
APPLICATION: US 2003-724292 A1 20031201 (10)

DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A recombinant adenoviral vector which contains an adenoviral genome from which the open reading frames E1 and/or E3 have been deleted, but retains enough sequence to make the adenoviral vector able to replicate in vitro, said vector also contains a therapeutic gene or a DNA sequence of interest regulated by ubiquitous promoters and/or tissue-specific promoters that encodes for therapeutic proteins useful in fibrosis treatment of the fibrosis.

2. The recombinant adenoviral vector according to claim 1, in which the specific tissue-promoter is PEPCK.

3. The recombinant adenoviral vector according to claim 1, in which the therapeutic gene or the DNA sequence cloned in such adenoviral vector is selected from latent and active human metalloprotease gene MMP-8, MMP-1, MMP-2, MMP-9 and MMP-13; human urokinase Plasminogen Activator gene (uPA wild type and/or modified), gene of the truncated receptor for TGF-β type II; and Smad 7 which encode for therapeutic proteins, that degrade excess of collagenic proteins deposited in the cirrhotic organs.

4. The recombinant adenoviral vector according to claim 3, in which the therapeutic gene is a DNA sequence selected from the gene of the Hepatocyte Growth Factor (HGF), which encodes for proteins stimulators of hepatic regeneration with the purpose to re-establish the normal functions of the liver.

5. The recombinant adenoviral vector according to claim 1, in which the therapeutic proteins for the treatment of fibrosis are the latent and/or active protein MMP-8, MMP-1, MMP-2, MMP-9 and MMP-13; uPA wild type and/or modified; the truncated receptor for TGF-β type II; betaglycan; HGF and Smad 7.

6. The recombinant adenoviral vector according to claim 1, which contains also the delivery of therapeutic genes or DNA sequences which encode for therapeutic proteins intended for the treatment of fibrosis in cirrhotic liver.

7. The recombinant adenoviral vector according to claim 6, in which the delivery of the therapeutic genes Is carried out in other organs with generalized fibrosis.

8. The recombinant adenoviral vector according to claim 7, in which the tissue-specific recognition of the therapeutic genes to the organs with fibrosis, is conducted by the administration route used.

9. The recombinant adenoviral vector according to claim 8, in which the administration route is endovenous.

10. The recombinant adenoviral vector according to claim 6, in which the organs with fibrosis are selected from liver, lung, heart, kidney, skin, and hypertrophic scars.

11. The recombinant adenoviral vector according to claim 10, in which the main target organ is the liver.

12. Recombinant adenoviral vectors according to claims 1 to 11 in which the delivery of therapeutic genes is realised through the use of viral or non viral vectors.

13. The recombinant adenoviral vector according to claim 12, in which non viral vectors are selected from plasmids and cationic and anionic liposomes.

14. The recombinant adenoviral vector according to claim 6, in which the efficient sending of collagenase gene MMP-8 to cirrhotic liver, can induce the degradation of collagen by means of over-expression of metalloproteases.

15. The recombinant adenoviral vector according to claim 1, characterized because it is used for the treatment of the hepatic, pulmonary, renal, heart fibrosis, keloids and hypertrophic scars, and which does not induce lethal toxicity.

16. A process to prepare recombinant adenoviral vectors through the cloning of reporter genes Lac-Z and GFP and the therapeutic gene, which encodes for therapeutic proteins for the treatment of hepatic, pulmonary, renal, and/or heart fibrosis, keloids and hypertrophic scars.

17. The process according to claim 16 in which the therapeutic gene is

MMP-1, MMP-2, MMP-9 and MMP-13; gene for human uPA wild type and/or modified; Smad 7 and gene of the truncated receptor of TGF-β type II.

18. The process according with claim 16 in which the recombinant adenoviral vector is pAdGFP-MMP-8.

19. A pharmaceutical composition containing a therapeutically effective amount with a regimen of unitary doses of viral particles of recombinant adenoviral vectors, according to claim 1, for the treatment of hepatic, pulmonary, renal, and heart fibrosis, keloids and hypertrophic scars, combined with a pharmaceutically compatible carrier.

20. The pharmaceutical composition according to claim 19, in which the unitary dose is of about 10^{7-1014} viral particles for an individual with fibrosis.

21. The use of recombinant adenoviral vector according with claim 1, for the elaboration of a bio-medication for the treatment of hepatic, pulmonary, renal, and heart fibrosis, keloids and hypertrophic scars.

L1 ANSWER 5 OF 8 USPATFULL on STN
2004:127471 Recombinant viral and non-viral vectors containing the human urokinase plasminogen activator gene and its utilization in the treatment of various types of hepatic, pulmonary, pancreatic and cardiac fibrosis and hypertrophic scars.
Borunda, Juan Armendariz, Col. Prado Coapa, MEXICO
Aguilar Cordova, **Estuardo**, Col. Prado Coapa, MEXICO
US 2004097455 A1 20040520
APPLICATION: US 2003-432989 A1 20031024 (10)
WO 2000-MX50 20001130
PRIORITY: MX 2000-11713 20001128
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1.- a process to prepare recombinant adenoviral and non-adenoviral vectors through the cloning of reporter genes and cDNA encoding for therapeutic proteins for the treatment of hepatic, pulmonary, renal, heart, pancreas fibrosis, keloids and hypertrophic scars, in mammals, including human beings.

2.- The process according to claim 1, in which the reporter gene is selected from bacterial beta-galactosidase gene (lac-Z).

3.- The process according to claim 1, in which the therapeutic gene is selected from the wild and/or modified human gene of urokinase derived plasminogen activator; and from the gene of the truncated receptor of TGF-β type II, coding for therapeutic proteins that degrade and/or prevent the synthesis and deposition of excess collagenic proteins deposited in the cirrhotic organs.

4.- The process according to claim 3 in which the protein coded for by the modified cDNA of the human gene of urokinase derived plasminogen activator huPA activates liver latent collagenases and matrix metalloproteases (MMP's) to promote the in situ degradation of excess of collagen components of the extracellular matrix (ECM) in the space of Disse or perisinusoid space.

5.- The process according to claim 3 in which the protein coded for by the cDNA of the human gene of urokinase derived plasminogen activator huPA, re-establishes also the functional liver mass upon inducing the remaining hepatocytes to replicate and thus repopulate the hepatic parenchyma to obtain the regeneration and cure of the damaged liver.

6.- The process according to claim 1, in which the therapeutic gene is a cDNA sequence selected from the hepatocyte growth factor HGF coding for protein stimulating liver regeneration in order to re-establish the normal functioning of liver and other organs affected by the same pathology.

7.- The process according to claim 6, in which liver regeneration, after the extracellular degradation (ECM) stimulated by the in situ activation of the gene of hepatocyte growth factor HGF was measured as the percentage of cells stained with a specific antibody against nuclear cellular proliferation antigen (PCNA).

8.- The process according to claim 7, in which the number of PCNA positive hepatocytes was ten times higher in the livers of rats treated with the recombinant adenoviral vector pAd.PGK-ΔNΔ-huPA than in normal livers and in the livers of rats treated with pAd-GFP and

9.- The process according to claim 7, in which labeling showed that about 55% of the hepatocytes were positively stained by anti-PCNA antibodies, both in periportal as well as in centrilobular areas two days after the injection of recombinant adenoviral vector pAd.PGK-ΔNΔC-huPA.

10.- The process according to claim 7, in which the positive cell detected by day 10 show the reestablishment of the liver functional mass, which was confirmed by the clear trend in liver functional tests such as AST, ALT, Bilirubin and prothrombin times.

11.- The process according to claim 1, in which viral recombinant vectors are selected from first, second, thirst and/or fourth generation adenoviral vector, "gutless", adenoviral vectors, retroviral vectors and adeno-associated vectors.

12.- The process according to claim 1, in which non viral recombinant vectors are constituted by phospholipidic components, liposomes of different structures and combined with different ligands for specific receptors.

13.- The process according to claim 12, in which non viral vectors are selected from plasmids and cationic and anionic liposomes.

14.- The process according to claim 1, in which viral and non viral recombinant vectors are prepared through the construction and coupling of cDNA of human gene of wild and/or modified urokinase derived plasminogen activator huPA, and from cDNA of the gene of TGF-β type II truncated receptor and from cDNA of hepatocyte growth factor HGF with plasmids from different origins and with regulatable and/or inducible promoters.

15.- The process according to claim 1, in which the recombinant adenoviral vector is pAd.PGK-ΔNΔC-huPA.

16.- The process according to claim 1, which also includes the delivery of therapeutic genes or DNA sequences coding for therapeutic proteins for fibrosis treatment in cirrhotic livers.

17.- The process according to claim 16, in which the delivery of therapeutic genes is conducted in other organs affected by generalized fibrosis.

18.- The process according to claim 16, in which the administration of therapeutic genes is also carried out through the tissue specific recognition of the therapeutic genes to the fibrotic organs through the administration route used and by the natural tropism to cirrhotic livers of the recombinant vector used.

19.- The process according to claim 18, in which the administration route is endovenous.

20.- The process according to claim 17, in which the fibrotic organs are selected from liver, lung, heart, kidney, pancreas, skin and hypertrophic scars, in mammals, including human beings.

21.- The process according to claim 20, in which the main target organ is liver.

22.- The process according to claim 16, in which the delivery of therapeutic genes is conducted through the use of viral or non viral vectors.

23.- The process according to claim 22, in which the viral vectors are selected from first, second, thirst and/or fourth generation adenoviral vectors, "gutless", adenoviral vectors, retroviral vectors and adeno-associated vectors.

24.- The process according to claim 22, in which non viral vectors are constituted by phospholipidic components, liposomes of different structures and combined with different ligands for specific receptors.

25.- The process according to claim 22, in which the viral and non-viral vectors are prepared through the construction and coupling of the cDNA of the human gene of wild and/or modified urokinase derived plasminogen activator huPA and the cDNA of the gene of TFG-beta type II truncated receptor with plasmids from different origins and with regulatable and/or inducible promoters.

26.- The process according to claim 24, in which the non-viral vectors

27.- The process according to claim 16, in which the efficient delivery of the human gene of urokinase derived plasminogen activator huPA to the cirrhotic liver can cause collagen degradation through metalloprotease over-expression.

28.- The process according to claims 16 to 27, characterized because it is used for the treatment of liver, lung, kidney, heart, pancreas fibrosis as well as keloids and hypertrophic scars.

29.- The process according to claim 1, in which the expression of huPA human therapeutic gene administrated to cirrhotic animals can be detected by ELISA essays and immunochemistry through the expression of the corresponding human protein.

30.- The process according to claim 1, in which the expression of the genes of type I, III and IV endogenous collagens was evaluated through semi-quantitative RT-PCR, which permitted the detection of specific changes in the baseline levels of said genes.

31.- The process according to claim 30, in which the expression of type I collagen dropped four times on day 10, the expression of type III collagen dropped about two times on day 10, and the expression of type IV collagen dropped about five times on day 6, in rats treated with the recombinant adenoviral vector pAd.PGK-ΔNΔC-huPA compared to rats treated with irrelevant adenovirus pAd-GFP.

32.- The process according to claim 31, in which huPA over-expression induces the turning off of the genes of type I, III and IV endogenous collagens coding for ECM proteins.

33.- A recombinant adenoviral vector obtained through the process according to claim 1, containing an adenoviral genome from which the open reading frames E1 and/or E3 have been deleted, but retaining enough sequence to make the adenoviral vector able to replicate in vitro, characterized also because said vector contains a therapeutic gene or a DNA sequence of interest regulated by ubiquitous promoters and/or tissue-specific promoters, or inducible and/or regulatable promoters, encoding for therapeutic proteins useful in fibrosis treatment.

34.- The recombinant adenoviral vector according to claim 33, in which the promoter is the promoter from cytomegalovirus (CMV).

35.- The recombinant adenoviral vector according to claim 33, in which the therapeutic gene or the DNA sequence cloned in such adenoviral vector is selected from the human gene of wild and/or modified huPA urokinase derived plasminogen activator and from the gene of TGF-beta type II truncated receptor encoding for therapeutic proteins that degrade and/or prevent the synthesis and deposition of excess collagenic proteins deposited in the cirrhotic organs.

36.- The recombinant adenoviral vector according to claim 33, in which the therapeutic gene is a DNA sequence selected from the gene of the Hepatocyte Growth Factor (HGF), which encodes for proteins stimulating hepatic regeneration with the purpose to re-establish the normal functions of the liver.

37.- The recombinant adenoviral vector according to claim 33, in which the therapeutic proteins for the treatment of fibrosis are the wild and/or modified huPA urokinase derived plasminogen activator; the TGF-beta type II truncated receptor and the hepatocyte growth factor HGF.

38- The recombinant adenoviral vector according to claim 33, which contains also the delivery of therapeutic genes or DNA sequences which encode for therapeutic proteins intended for the treatment of fibrosis in cirrhotic liver.

39.- The recombinant adenoviral vector according to claim 38, in which the delivery of the therapeutic genes is carried out in other organs with generalized fibrosis.

40.- The recombinant adenoviral vector according to claim 38, in which the delivery of therapeutic genes is also conducted through the tissue-specific recognition of the therapeutic genes to the fibrotic organs by the administration route used and by natural tropism to cirrhotic liver of the recombinant vectors used.

41.- The recombinant adenoviral vector according to claim 40, in which the administration route is endovenous.

42.- The recombinant adenoviral vector according to claim 39, in which

pancreas, skin, and hypertrophic scars, in mammals, including human beings.

43.- The recombinant adenoviral vector according to claim 42, in which the main target organ is the liver.

44.- The recombinant adenoviral vector according to claims 33 to 43, which is pAd.PGK-ΔNΔC-huPA.

45.- The recombinant vector according to claim 38, in which the sending of therapeutic genes is conducted through the use of viral and non-viral vectors.

46.- The recombinant vector according to claim 45, in which the viral vectors are selected from first, second, third and/or fourth generation of adenoviral vectors, "gutless" adenoviral vectors and adeno-associated vectors.

47.- The recombinant vector according to claim 45, in which the non-viral vectors can be constituted by phospholipidic components; liposomes from different structures and combined with different ligands for specific receptors.

48.- The recombinant vector according to claim 45, in which the viral and non-viral vectors are prepared through the construction and coupling of the cDNA of the human gene of wild and/or modified urokinase derived plasminogen activator and the cDNA of the TGF-beta type truncated receptor with plasmids from different origins and with regulatable and/or inducible promoters.

49.- The recombinant adenoviral vector according to claim 47, in which the non viral vectors are selected from plasmids and cationic and anionic liposomes.

50.- The recombinant adenoviral vector according to claim 38, in which the efficient delivery of the human gene of urokinase derived plasminogen activator huPA to liver can induce collagen degradation by means of over-expression of metalloproteases.

51.- The recombinant adenoviral vector according to claims 33 to 50, characterized because it is used for the treatment of the hepatic, pulmonary, renal, heart, pancreatic fibrosis, keloids and hypertrophic scars, and does not induce lethal toxicity in mammals, including human beings.

52.- The recombinant adenoviral vector according to claims 33 to 51, in which the additional advantage of using said recombinant vectors is that, because they do not secrete significant amount of huPA, they do not cause hypercoagulation or spontaneous bleeding, the main disadvantage in cirrhotic animals.

53.- The recombinant adenoviral vector according to claim 52, in which a non secreted huPA form with retention signal modification in endoplasmic reticulum (RE) at the amino-terminus and carboxi-terminus is used in order to prevent as much as possible its secretion in the blood stream.

54.- The recombinant adenoviral vector according to claim 53, in which cDNA of huPA cloned in pGEM3 in Xba I/Asp 718 sites is modified at the carboxi-terminus adding a sequence coding for KDEL signal, and also residues upstream through the cloning of 75 PCR-generated nucleotides.

55.- The recombinant adenoviral vector according to claim 54, in which the amino-terminal 25 amino acids that include the pre-uPA peptidic signal were substituted by the amino-terminal retention signal (RR) next to the anchor in the transmembrane region (TM) separated by a spacer peptide of 31 amino acids from transmembrane II protein Iip33 obtained by PCR.

56.- The recombinant adenoviral vector according to claim 55, in which the RR sequence is constituted by arginine residue MHRRRSR located next to the anchor region of transmembrane (TM) and are present in type II transmembrane proteins such as the invariable chain protein Iip33.

57.- The recombinant adenoviral vector according to claims 53, in which the modified huPA gene is cloned on the adenoviral plasmid pAd.ΔNΔC-huPA for recombinant adenoviral vector production.

58.- A pharmaceutical composition containing a therapeutically effective quantity, with a unitary dose regimen, of viral particles of the recombinant adenoviral vectors according to claims 33 to 57, for the treatment of liver, lung, kidney, heart, pancreas fibrosis, keloids and hypertrophic scars, combined with a pharmaceutically acceptable carrier.

59.- The pharmaceutical composition according to claim 58, in which the unitary dose contains about 10^{7-1014} viral particles per fibrotic individual.

60.- The use of recombinant adenoviral vectors, and other viral and non-viral vectors according to claims 33 to 57, to prepare a drug containing a therapeutically effective quantity, with a unitary dose regimen, of viral particles for the treatment of liver, lung, kidney, heart, pancreas fibrosis, keloids and hypertrophic scars, in mammals, including human beings.

61.- The use according to claim 60, in which the unitary dose contains about 10^{7-1014} viral particles per fibrotic individual.

62.- A method for the treatment of treatment of liver, lung, kidney, heart, pancreas fibrosis, keloids and hypertrophic scars, in mammals, including human beings, consisting in the administration of a recombinant adenoviral vector according to claims 33 to 57.

63.- A method for the treatment of liver, lung, kidney, heart, pancreas fibrosis, keloids and hypertrophic scars, in mammals, including human beings, consisting in the administration of the pharmaceutical composition according to claims 58 to 59.

64.- The method according to claim 62 or 64, in which the route of administration is endovenous.

65.- The method according to claim 62 or 63, in which the unitary dose contains about 10^{7-1014} viral particles per fibrotic individual.

L1 ANSWER 6 OF 8 USPATFULL on STN
2004:113648 Chemeric viral vectors for gene therapy.
Aguilar-Cordova, Carlos Estuardo, Newton, MA, UNITED STATES
US 2004086485 A1 20040506
APPLICATION: US 2002-264839 A1 20021004 (10)
PRIORITY: US 2001-327179P 20011004 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. Method of delivery of a therapeutic genetic molecule to target tissue comprising delivery of a non-viral form of the genetic molecule with a precursor of in-vivo viral vector production or as such a precursor.

2. Method in accordance with claim 1 wherein a nucleic acid sequence is delivered in a plasmid form comprising all the necessary elements for the production of a viral vector.

3. Method in accordance with claim 2 wherein the plasmide is directed to specific targetted tissues by the addition of conjugated molecules.

4. Method in accordance with claim 3 with the conjugated molecules being selected from the group consisting of polycations, peptides, antibodies, single chain antibodies and combinations of two or more of them.

5. Method in accordance with claim 1 wherein the nucleic acid sequence contains the necessary sequences for production of a replication competent virus.

6. Method in accordance with claim 1 wherein a nucleic acid sequence comprises the whole adenoviral genome and wherein the regulatory elements of the virus, such as the E1 genes, are under the regulatory control of tissue associated sequences.

7. Method in accordance with claim 1 wherein the control of gene expresson is mediated by post-transcriptional or post-translational tissue effects, such as the permissivity for intron excission or complex enzyme formation.

8. Method in accordance with claim 1 wherein a nucleic acid region for targeting an adenoviral vector is provided as the precursor.

9. Method in accordance with claim 1 wherein an additional DNA sequence (additional to the therapeutic genetic molecule) is provided and wherein said additional sequence contains retroviral long terminal repeat flanking regions flanking a cassette, wherein said cassette contains a nucleic acid region of interest.

10. Method in accordance with claim 9 wherein the cassette content is selected from the group consisting of a gag nucleic acid region; a pol nucleic acid sequence; and a sequence capable of providing the

or the vesicular stomatitis G protein (VSV-G).

11. Method in accordance with claim 1 wherein a nucleic acid sequence as described above and and a nucleic acid region for targeting a retroviral vector is provided in addition to the therapeutic gene sequence.

12. Method in accordance with claim 2 wherein the plasmid sequence for in-vivo delivery is comprised of sequences necessary for other replication competent or conditional viruses, such as picorna viruses, alpha viruses, herpes viruses, parvoviruses, rhinoviruses, baculoviruses.

13. Method in accordance with claim 12 and further comprising as part of the delivery a suicide nucleic acid region.

14. The method of claim 1 wherein the delivery comprises a transactivator nucleic acid region located in the construct to regulate gene expression.

15. Method in accordance with claim 14 wherein the transactivator is the tetracycline transactivator.

16. Method in accordance with claim 1 wherein the expression of an env nucleic acid region is provided to regulate an inducible promoter nucleic acid region.

17. Method in accordance with claim 16 wherein the inducible promoter nucleic acid region is induced by a stimulus selected from the group consisting of tetracycline, galactose, glucocorticoid, Ru487 and heat shock.

18. Method in accordance with claim 1 wherein an env nucleic acid region is provided that is selected from the group consisting of amphotropic envelope, xenotropic envelope, ecotropic envelope, human immunodeficiency virus 1 (HIV-1) envelope, human immunodeficiency virus 2 (HIV-2) envelope, feline immunodeficiency virus (FIV) envelope, simian immunodeficiency virus 1(SIV) envelope, human T-cell leukemia virus 1 (HTLV-1) envelope, human T-cell leukemia virus 2 (HTLV-2) envelope and vesicular stomatis virus-G glycoprotein.

19. Method in accordance with claim 13 wherein the suicide nucleic acid region is selected from the group consisting of Herpes simplex virus type 1 thymidise kinase, oxidoreductase, cytosine deaminase, thymidine kinase thymidilate kinase (Tdk::Tmk) and deoxycytidine kinase.

20. The method of claim 1 wherein there is provided with the therapeutic gene a plasmid comprising the retroviral long terminal repeat flanking regions flanking a cassette, wherein said cassette nucleic acid region of interest, the plasmid further containing a gag nucleic acid region; a pol nucleic acid region; and a nucleic acid region from the group consisting of an env nucleic acid region, a nucleic acid region for pseudotyping a retroviral vector.

21. Method in accordance with claim 1 wherein the chimeric nucleic acid plasmid further comprises a suicide nucleic acid.

22. Method in accordance with claim 21 wherein the plasmid further comprises a transactivator nucleic acid region, wherein said transactivator nucleic acid region encodes a polypeptide which regulates transcription of an env nucleic acid region.

23. Method in accordance with claim 1 wherein a nucleic acid vector comprising the adeno-associated viral terminal repeat flanking regions flanking a cassette is provided and wherein said cassette contains a nucleic acid region of therapeutic interest, the plasmid further containing a rep nucleic acid region; a cap nucleic acid region; and an adenoviral E1 and E4 nucleic acid region.

24. Method in accordance with claim 1 wherein the further component comprises an env polypeptide selected from the group consisting of amphotropic envelope, xenotropic envelope, ecotropic envelope, human immunodeficiency virus 1 (HIV-1) envelope, human immunodeficiency virus 2 (HIV-2) envelope, feline immunodeficiency virus (FIV) envelope, simian immunodeficiency virus 1(SIV) envelope, human T-cell leukemia virus 1 (HTLV-1) envelope, human T-cell leukemia virus 2 (HTLV-2) envelope and vesicular stomatis virus-G glycoprotein.

25. Method in accordance with claim 1 wherein the further component comprises a sequence intervening a functional gene that is excised when complemented in the target tissue to form a functional self splicing intron.

hepatocyte.

27. Method in accordance with claim 1 wherein the therapeutic nucleic acid region of is selected from the group consisting of a reporter region, ras, myc, raf, erb, src, fins, jun, trk, ret, gsp, hst, bcl abl, Rb, CFTR, pl6, p21, p27, p53, p57, p73, C-CAM, APC, CTS-1, zacl, scFV ras, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, BRCAl, VHL, MMACl, FCC, MCC, BRCA2, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11 IL-12, GM-CSF G-CSF, thymidine kinase, CD40L, Factor VIII, Factor IX, CD40, multiple disease resistance (MDR), ornithine transcarbamylase (OTC), ICAM-1, HER2-neu, PSA, terminal transferase, caspase, NOS, VEGF, FGF, bFGF, HIS, heat shock proteins, IFN alpha and gamma, TNF alpha and beta, telomerase, and insulin receptor.

L1 ANSWER 7 OF 8 USPATFULL on STN
2003:268143 Aldehyde reductase bidirectional promoter and its use.
Barski, Oleg A., Houston, TX, United States
Aguilar-Cordova, **Estuardo C.**, Newton, MA, United States
Bohren, Kurt M., Pearland, TX, United States
Gabbay, Kenneth H., Houston, TX, United States
Baylor College of Medicine, Houston, TX, United States (U.S. corporation)
US 6630324 B1 20031007
APPLICATION: US 2000-626002 20000726 (9)
PRIORITY: US 1999-146266P 19990729 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

CLM What is claimed is:
1. A DNA sequence of the formula selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:30, and SEQ ID NO:31, or fragments thereof; wherein said fragments function as bidirectional promoters.

2. A promoter having the characteristic of promoting transcription of two separate nucleotide sequences, wherein one of such nucleotide sequences is operatively linked to the 5' end of said promoter sequence and is transcribed 5' to 3' in the direction opposite from the 5' to 3' direction of said promoter sequence and the other of such nucleotide sequences is operatively linked to the 3' end of said promoter sequence and is transcribed 5' to 3' in the same direction as the 5' to 3' direction of said promoter sequence, wherein the promoter promotes transcription of the two nucleotide sequences in approximately equimolar amounts, and wherein the promoter sequence is selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:29; SEQ ID NO:30, SEQ ID NO:31 and fragments thereof wherein said fragments function as bidirectional promoters.

3. A recombinant DNA vector comprising: the promoter sequence selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:29; SEQ ID NO:30 and SEQ ID NO:31 or fragments thereof, wherein said fragments function as a bidirectional promoter; a first DNA sequence encoding a gene operatively linked to the 5' end of the promoter sequence wherein when said first DNA sequence is transcribed it is transcribed 5' to 3' in the direction opposite from the 5' to 3' direction of the promoter sequence; and a second DNA sequence encoding a gene operatively linked to the 3' end of the promoter sequence wherein when said second DNA sequence is transcribed it is transcribed 5' to 3' in the same direction as the 5' to 3' direction of the promoter sequence.

4. The recombinant DNA vector of claim 3, wherein said first DNA sequence and said second DNA sequence encode nonidentical nucleotide sequences.

5. The recombinant DNA vector of claim 3, wherein one of said DNA sequences encodes a reporter sequence.

6. The recombinant DNA vector of claim 5, wherein the reporter sequence is selected from the group consisting of ampicillin, neomycin, kanamycin, luciferase, β-galactosidase, β-glucuronidase, chloramphenicol acetyl-transferase (CAT), blue fluorescent protein (BFP), green fluorescent protein (GFP), and placental alkaline phosphatase.

7. The recombinant DNA vector of claim 3, wherein one of the DNA sequences encodes a heavy chain of an antibody and the other DNA sequence encodes a light chain of said antibody.

8. The method of preparing an antibody containing a light chain and a

encoded for by the DNA sequences in the recombinant DNA vector of claim 7 comprising: (a) expression of said DNA sequences, wherein said heavy and light chain sequences combine to form an antibody; and (b) recovering the formed antibody.

9. The recombinant DNA vector of claim 3, wherein one DNA sequence encodes the p35 subunit of Interleukin-12 and the other DNA sequence encodes the p40 subunit of Interleukin-12.

10. A method of preparing Interleukin-12 containing p35 and p40 subunits of said Interleukin-12 wherein said p35 and p40 subunits are encoded for by the DNA sequences in the recombinant DNA vector of claim 9 comprising: (a) expression of said DNA sequences, wherein said subunit sequences combine to form Interleukin-12; and (b) recovering the formed Interleukin-12.

11. The recombinant DNA vector of claim 3, wherein one DNA sequence encodes a subunit of Interleukin-2 and the other DNA sequence encodes the other subunit of Interleukin-2.

12. A method of preparing Interleukin-2 containing subunits of said Interleukin-2 wherein said subunits are encoded for by the DNA sequences in the recombinant DNA vector of claim 11 comprising: (a) expression of said DNA sequences, wherein said subunit sequences combine to form Interleukin-2; and (b) recovering the formed Interleukin-2.

13. The recombinant DNA vector of claim 3 wherein said DNA sequences each encode growth hormone receptor subunits.

14. A method of preparing the growth hormone receptor containing the subunits of said growth hormone receptor wherein said subunits are encoded for by the DNA sequences in the recombinant DNA vector of claim 13 comprising: (a) expression of said DNA sequences, wherein said subunit sequences combine to form a growth hormone receptor; and (b) recovering the formed growth hormone receptor.

15. The recombinant DNA vector of claim 3 wherein at least one of said DNA sequences encodes a subunit of a homodimer.

16. A method of preparing a homodimer containing the subunit of said homodimer wherein said subunit is encoded for by the DNA sequences in the recombinant DNA vector of claim 15 comprising: (a) expression of said DNA sequences, wherein said subunit sequences combine to form a homodimer; and (b) recovering the formed homodimer.

17. The recombinant DNA vector of claim 3 wherein one DNA sequence encodes one subunit of a heterodimer and the other DNA sequence encodes another subunit of said heterodimer.

18. A method of preparing a heterodimer containing the subunits of said heterodimer wherein said subunits are encoded for by the DNA sequences in the recombinant DNA vector of claim 17 comprising: (a) expression of said DNA sequences, wherein said subunit sequences combine to form a heterodimer; and (b) recovering the formed heterodimer.

19. The recombinant DNA vector of claim 3 wherein at least one nucleotide sequence encodes a RNA; said RNA being the final product of said nucleotide sequence.

20. The recombinant DNA vector of claim 19 wherein said RNA is selected from the group comsisting of telomerase RNA, snRNA, snoRNA, scRNA, antisence RNA and XIST RNA.

21. The method of preparing a ribonucleoprotein containing an RNA and a protein each encoded by one of the DNA sequences of the recombinant DNA vector of claim 3 comprising: (a) expression of said DNA sequences, wherein said RNA and protein sequences combine to form a ribonucleoprotein; and (b) recovering the formed ribonucleoprotein.

22. A recombinant DNA vector comprising: the promoter sequence selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:29; SEQ ID NO:30 and SEQ ID NO:31 or fragments thereof, wherein said fragments function as a bidirectional promoter; a first polylinker site into which a first DNA sequence is operatively linked to the 5' end of the promoter sequence, wherein when said first DNA sequence is transcribed it is transcribed 5' to 3' in the direction opposite from the 5' to 3' direction of the promoter sequence; and a second polylinker site into which a second DNA sequence encoding a gene operatively linked to the 3' end of the promoter sequence wherein when said second DNA sequence is transcribed it is transcribed 5' to 3' in the same direction as the 5' to 3' direction of the promoter sequence.

23. The recombinant DNA vector of claim 3, wherein said vector further comprises a cassette, wherein said cassette comprises one DNA sequence which encodes a suicide nucleic acid sequence and the other DNA sequence which encodes an immortalization nucleic acid sequence, wherein both DNA sequences are transcribed from the promoter sequence.

24. The vector of claim 23, wherein said suicide nucleic acid sequence is thymidine kinase (TK).

25. The vector of claim 23, wherein said immortalization nucleic acid sequence is selected from the group consisting of T-antigen, telomerase catalytic protein subunit and myc.

26. A method to initiate proliferation of a cell comprising the step of introducing the vector of claim 23 into said cell under conditions wherein activation of the bidirectional promoter to transcribe said immortalization nucleic acid sequence in said cell initiates proliferation of said cell.

27. The recombinant DNA vector of claim 23, wherein said vector further comprises excision sites flanking said cassette, wherein said excision sites are selected from the group consisting of lox, FLP recognition target sites, restriction endonuclease sites, and transposon sequences.

28. A method to initiate proliferation of a cell comprising the step of introducing the vector of claim 27 into said cell under conditions wherein activation of the bidirectional promoter to transcribe said immortalization nucleic acid sequence in said cell initiates proliferation of said cell.

29. The method of claim 26 or 28, wherein said primary cell is selected from the group consisting of insulin-producing beta cell and liver cell.

30. The method of claim 26 or 28, wherein said cell is a primary cell.

31. The method of claim 28, wherein said introduction step further comprises integration of said excision sites and said cassette into a provirus of said cell.

32. The method of claim 31, wherein said method further comprises excising said immortalization nucleic acid sequence from said provirus through said excision sites.

33. The method of claim 32, wherein said method further comprises destroying a cell which has failed to excise said immortalization nucleic acid sequence.

34. The recombinant DNA vector of claim 3 or 22 further comprising a poly A+ polyadenylation sequence operatively linked to the 3' end of at least one of said first or second DNA sequences.

L1 ANSWER 8 OF 8 USPATFULL on STN
2003:3043 Recombinant adenoviral vectors and their utility in the treatment of various types of fibrosis: hepatic, renal, pulmonary, as well as hypertrophic scars.
Armendariz Borunda, Juan, Prado Coapa, MEXICO
Aguilar Cordova, Estuardo, Col. Prado Coapa, MEXICO
US 2003003077 A1 20030102
APPLICATION: US 2002-98359 A1 20020318 (10)
PRIORITY: MX 1999-998515 19990917
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1.- A recombinant adenoviral vector which contains an adenoviral genome from which the open reading frames E1 and/or E3 have been deleted, but retains enough sequence to make the adenoviral vector able to replicate in vitro, said vector also contains a therapeutic gene or a DNA sequence of interest regulated by ubiquitous promoters and/or tissue-specific promoters that encodes for therapeutic proteins useful in fibrosis treatment of the fibrosis.

2.- The recombinant adenoviral vector according to claim 1, in which the specific tissue-promoter is PEPCK.

3.- The recombinant adenoviral vector according to claim 1, in which the therapeutic gene or the DNA sequence cloned in such adenoviral vector is selected from latent and active human metalloprotease gene MMP-8, MMP-1, MMP-2, MMP-9 and MMP-13; ; human urokinase Plasminogen Activator gene (uPA wild type and/or modified), gene of the truncated receptor for TGF-β type II; and Smad 7 which encode for therapeutic proteins, that degrade excess of collagenic proteins deposited in the cirrhotic

4.- The recombinant adenoviral vector according to claim 3, in which the therapeutic gene is a DNA sequence selected from the gene of the Hepatocyte Growth Factor (HGF), which encodes for proteins stimulators of hepatic regeneration with the purpose to re-establish the normal functions of the liver.

5.- The recombinant adenoviral vector according to claim 1, in which the therapeutic proteins for the treatment of fibrosis are the latent and/or active protein MMP-8, MMP-1, MMP-2, MMP-9 and MMP-13; uPA wild type and/or modified; the truncated receptor for TGF-β type II; betaglycan; HGF and Smad 7.

6.- The recombinant adenoviral vector according to claim 1, which contains also the delivery of therapeutic genes or DNA sequences which encode for therapeutic proteins intended for the treatment of fibrosis in cirrhotic liver.

7.- The recombinant adenoviral vector according to claim 6, in which the delivery of the therapeutic genes is carried out in other organs with generalized fibrosis.

8.- The recombinant adenoviral vector according to claim 7, in which the tissue-specific recognition of the therapeutic genes to the organs with fibrosis, is conducted by the administration route used.

9.- The recombinant adenoviral vector according to claim 8, in which the administration route is endovenous.

10.- The recombinant adenoviral vector according to claim 6, in which the organs with fibrosis are selected from liver, lung, heart, kidney, skin, and hypertrophic scars.

11.- The recombinant adenoviral vector according to claim 10, in which the main target organ is the liver.

12.- Recombinant adenoviral vectors according to claims 1 to 11 in which the delivery of therapeutic genes is realised through the use of viral or non viral vectors.

13.- The recombinant adenoviral vector according to claim 12, in which non viral vectors are selected from plasmids and cationic and anionic liposomes.

14.- The recombinant adenoviral vector according to claim 6, in which the efficient sending of collagenase gene MMP-8 to cirrhotic liver, can induce the degradation of collagen by means of over-expression of metalloproteases.

15.- The recombinant adenoviral vector according to claim 1, characterized because it is used for the treatment of the hepatic, pulmonary, renal, heart fibrosis, keloids and hypertrophic scars, and which does not induce lethal toxicity.

16.- A process to prepare recombinant adenoviral vectors through the cloning of reporter genes Lac-Z and GFP and the therapeutic gene, which encodes for therapeutic proteins for the treatment of hepatic, pulmonary, renal, and/or heart fibrosis, keloids and hypertrophic scars.

17.- The process according to claim 16 in which the therapeutic gene is selected from the human metalloprotease gene MMP-8 latent and active, MMP-1, MMP-2, MMP-9 and MMP-13; gene for human uPA wild type and/or modified; Smad 7 and gene of the truncated receptor of TGF-β type II.

18.- The process according with claim 16 in which the recombinant adenoviral vector is pAdGFP-MMP-8.

19.- A pharmaceutical composition containing a therapeutically effective amount with a regimen of unitary doses of viral particles of recombinant adenoviral vectors, according to claim 1, for the treatment of hepatic, pulmonary, renal, and heart fibrosis, keloids and hypertrophic scars, combined with a pharmaceutically compatible carrier.

20.- The pharmaceutical composition according to claim 19, in which the unitary dose is of about 10^{7-1014} viral particles for an individual with fibrosis.

21.- The use of recombinant adenoviral vector according with claim 1, for the elaboration of a bio-medication for the treatment of hepatic, pulmonary, renal, and heart fibrosis, keloids and hypertrophic scars.

```
=> e belmont john w/in
E1            1     BELMONT JAMES E/IN
E2            1     BELMONT JEAN MARC/IN
E3            3 --> BELMONT JOHN W/IN
E4            5     BELMONT KIRK E/IN
E5            1     BELMONT LEE/IN
E6            1     BELMONT MICHAEL RICHARD/IN
E7            1     BELMONT NORMAN J/IN
E8            3     BELMONT PETER A/IN
E9            1     BELMONT PIERRE/IN
E10           2     BELMONT RICHARD/IN
E11          10     BELMONT RICHARD E/IN
E12           8     BELMONT STEPHEN E/IN

=> s e3
L2            3 "BELMONT JOHN W"/IN

=> s l2 not l1
L3            2 L2 NOT L1

=> d l3,ti,1-2

L3   ANSWER 1 OF 2  USPATFULL on STN
TI     Phosphatases which activate map kinase pathways

L3   ANSWER 2 OF 2  USPATFULL on STN
TI     Phosphatases which activate map kinase pathways


=> e harper j wade/in
E1            1     HARPER IV WILLIAM M/IN
E2            1     HARPER J CLETUS/IN
E3            1 --> HARPER J WADE/IN
E4            1     HARPER J WILLIAM/IN
E5            2     HARPER JACK/IN
E6            3     HARPER JACK D/IN
E7            6     HARPER JACK R/IN
E8            1     HARPER JACQUE LUCILLE/IN
E9            5     HARPER JAMES/IN
E10           1     HARPER JAMES A/IN
E11           5     HARPER JAMES B/IN
E12           8     HARPER JAMES C/IN

=> s e3
L4            1 "HARPER J WADE"/IN

=> s l4 not l1
L5            0 L4 NOT L1

=> file wpids
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                               23.15      23.36

FILE 'WPIDS' ENTERED AT 08:26:29 ON 27 JUN 2006
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FILE LAST UPDATED:          23 JUN 2006      <20060623/UP>
MOST RECENT DERWENT UPDATE:     200640        <200640/DW>
DERWENT WORLD PATENTS INDEX SUBSCRIBER FILE, COVERS 1963 TO DATE

>>> FOR A COPY OF THE DERWENT WORLD PATENTS INDEX STN USER GUIDE,
    PLEASE VISIT:
 http://www.stn-international.de/training_center/patents/stn_guide.pdf <

>>> FOR DETAILS OF THE PATENTS COVERED IN CURRENT UPDATES, SEE
http://scientific.thomson.com/support/patents/coverage/latestupdates/

>>> PLEASE BE AWARE OF THE NEW IPC REFORM IN 2006, SEE
http://www.stn-international.de/stndatabases/details/ipc_reform.html and
http://scientific.thomson.com/media/scpdf/ipcrdwpi.pdf <<<

>>> FOR FURTHER DETAILS ON THE FORTHCOMING DERWENT WORLD PATENTS
    INDEX ENHANCEMENTS PLEASE VISIT:
http://www.scientific.thomson.com/cm/dwpienhancements <<<

=> e aguilar cordova c e/in
E1            1     AGUILAR CHAVEZ C/IN
E2            1     AGUILAR CORDOBA C E/IN
E3            4 --> AGUILAR CORDOVA C E/IN
E4            4     AGUILAR CORDOVA E/IN
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E6              1      AGUILAR CORTES F/IN
E7              1      AGUILAR COVO R/IN
E8              5      AGUILAR D/IN
E9              7      AGUILAR D A/IN
E10             1      AGUILAR D G/IN
E11             3      AGUILAR DE KEGEL D/IN
E12             2      AGUILAR DIEZ E/IN

=> s e2-e5
                1 "AGUILAR CORDOBA C E"/IN
                4 "AGUILAR CORDOVA C E"/IN
                4 "AGUILAR CORDOVA E"/IN
                1 "AGUILAR CORDOVA E C"/IN
L6              9 ("AGUILAR CORDOBA C E"/IN OR "AGUILAR CORDOVA C E"/IN OR "AGUILA
                  R CORDOVA E"/IN OR "AGUILAR CORDOVA E C"/IN)

=> d l6,ti,1-9

L6   ANSWER 1 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   New composition comprising one or more replication conditional (RC)
     adenoviral vectors and one or more replication defective (RD) adenoviral
     viral vectors, useful for treating cancer.

L6   ANSWER 2 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   New bidirectional promoter, useful e.g. for recombinant production of
     antibodies or other subunit proteins, provides equal levels of expression
     of two nucleic acids.

L6   ANSWER 3 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Delivering a therapeutic genetic molecule to a target tissue for treating
     e.g. immunodeficiency syndromes or cancer by delivering a non-viral form
     of the genetic molecule with a precursor of in-vivo viral vector
     production.

L6   ANSWER 4 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Preparing recombinant vector containing reporter and therapeutic genes,
     useful for treatment of fibrosis, particularly of liver, by inducing
     degradation of collagen.

L6   ANSWER 5 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   New chimeric nucleic acid vectors comprising adenoviral inverted terminal
     repeat flanking regions, and an internal region between the adenoviral
     flanking regions, useful for delivering therapeutic genes into a cell, or
     gene therapy.

L6   ANSWER 6 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Solid tumor treatment comprising gene therapy combined with e.g. radiation
     therapy or chemotherapy.

L6   ANSWER 7 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Recombinant adenoviral vector, useful for treatment of fibrosis, comprises
     replicable viral genome and therapeutic gene, e.g. for matrix
     metalloprotease 8.

L6   ANSWER 8 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Non-integrating (adenovirus-based) viral vectors useful in gene therapy,
     especially for treating patients suffering from a genetic disease, e.g.
     cystic fibrosis, diabetes, cardiovascular disease, cancer or brain
     malfunction.

L6   ANSWER 9 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   New double trans-dominant mutant gene contg. both rev and tat mutants -
     and related protein, inhibits two essential replicative functions
     simultaneously, useful for treatment of HIV infection.


=> e belmont j w/in
E1              1      BELMONT J E/IN
E2              3      BELMONT J M/IN
E3              2 --> BELMONT J W/IN
E4              4      BELMONT K E/IN
E5              1      BELMONT L/IN
E6              3      BELMONT M R/IN
E7              8      BELMONT P/IN
E8              2      BELMONT R/IN
E9              4      BELMONT R E/IN
E10             7      BELMONT S/IN
E11             8      BELMONT S E/IN
E12             2      BELMONTE A F/IN

=> s e3
L7              2 "BELMONT J W"/IN
```

```
=> s l7 not l6
L8             1 L7 NOT L6

=> d l8,ti

L8   ANSWER 1 OF 1  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   New human or mouse c-Jun amino-terminal kinase (JNK) activating
     phosphatase is useful for diagnosing or treating diseases associated with
     JNK activating phosphatase or JNK-mediated disorders, e.g. inflammatory
     diseases.


=> e harper j w/in
E1             7     HARPER J S/IN
E2             2     HARPER J T/IN
E3            10 --> HARPER J W/IN
E4             3     HARPER JONES F D/IN
E5             8     HARPER K/IN
E6             6     HARPER K A/IN
E7             2     HARPER K B/IN
E8             3     HARPER K D/IN
E9             4     HARPER K E/IN
E10            1     HARPER K E C/IN
E11            2     HARPER K K/IN
E12           12     HARPER K L/IN

=> s e3
L9            10 "HARPER J W"/IN

=> s l9 not l6
L10            9 L9 NOT L6

=> d l10,ti,1-9

L10  ANSWER 1 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Novel isolated nucleotide sequence encoding functionally active fragment
     of F-box protein, useful in detecting nuclear factor kappa B regulatory
     factors.

L10  ANSWER 2 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   New isolated nucleic acid segment encoding a protein with at least one
     functionally active F-box domain, useful for identifying related genes,
     and for developing compounds for treating infectious or inflammatory
     disease.

L10  ANSWER 3 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Database administration and replication method involves storing statistics
     for each of database sampled records to perform extrapolated replication
     partition analysis operation on database.

L10  ANSWER 4 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Database partition boundary determination method in information system,
     involves sampling records of database using random number algorithms,
     which are added or deleted from database.

L10  ANSWER 5 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Detecting nuclear factor-kappaB regulatory factors, such as F-box proteins
     involved in targeted ubiquitination, by contacting the regulatory factors
     with slimb protein to form a complex and detecting the complex.

L10  ANSWER 6 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   New isolated F-box proteins and genes for development of therapeutics,
     e.g. for cancer treatment.

L10  ANSWER 7 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   New mutant angiogenin derivs. having altered 116 aminoacid - with higher
     angiogenic and ribonucleolytic activities, used for promoting development
     of blood vessels, etc..

L10  ANSWER 8 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Mitogenic polypeptide isolated from human brain tissue - useful for
     increasing vascular effect in e.g. wound healing, or generating
     endothelial cell linings for vascular prostheses, etc..

L10  ANSWER 9 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Cargo tie-down device - comprises channel and rod attached to channel and
     at least one track recessed within securing surface.


=> file uspatful
COST IN U.S. DOLLARS                    SINCE FILE      TOTAL
                                           ENTRY    SESSION
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FILE 'USPATFULL' ENTERED AT 08:27:55 ON 27 JUN 2006
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FILE COVERS 1971 TO PATENT PUBLICATION DATE: 27 Jun 2006 (20060627/PD)
FILE LAST UPDATED: 27 Jun 2006 (20060627/ED)
HIGHEST GRANTED PATENT NUMBER: US7069595
HIGHEST APPLICATION PUBLICATION NUMBER: US2006137066
CA INDEXING IS CURRENT THROUGH 27 Jun 2006 (20060627/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 27 Jun 2006 (20060627/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Feb 2006
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Feb 2006


=> e aguilar cordova c e/au
E1            2     AGUILAR CARLOS L/AU
E2            1     AGUILAR CARMEN ARNALOT I/AU
E3            0 --> AGUILAR CORDOVA C E/AU
E4            1     AGUILAR CORDOVA C ESTUARDO/AU
E5            1     AGUILAR CORDOVA CARLOS ESTUARDO/AU
E6            5     AGUILAR CORDOVA ESTUARDO/AU
E7            1     AGUILAR CORDOVA ESTUARDO C/AU
E8            2     AGUILAR CORINNE/AU
E9            6     AGUILAR DANIEL A/AU
E10           1     AGUILAR DANIEL ALFONSO/AU
E11           2     AGUILAR DAVID G/AU
E12           1     AGUILAR DENNIS/AU

=> file medline
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     1.43      33.27

FILE 'MEDLINE' ENTERED AT 08:28:23 ON 27 JUN 2006

 FILE LAST UPDATED: 24 JUN 2006 (20060624/UP).  FILE COVERS 1950 TO DATE.

 On December 11, 2005, the 2006 MeSH terms were loaded.

 The MEDLINE reload for 2006 is now (26 Feb.) available.  For details
 on the 2006 reload, enter HELP RLOAD at an arrow prompt (=>).
 See also:

    http://www.nlm.nih.gov/mesh/
    http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
    http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
    http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

 OLDMEDLINE is covered back to 1950.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2006 vocabulary.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> e aguilar cordova c e/au
E1            1     AGUILAR CORDERO M JOSE/AU
E2            1     AGUILAR CORDOBA E/AU
E3            1 --> AGUILAR CORDOVA C E/AU
E4           57     AGUILAR CORDOVA E/AU
E5            1     AGUILAR CORDOVA ESTAURDO/AU
E6           17     AGUILAR CORDOVA ESTUARDO/AU
E7            6     AGUILAR CORTES E/AU
E8            2     AGUILAR CORTES F/AU
E9            1     AGUILAR CORTES M/AU
E10           2     AGUILAR COTA M E/AU
E11           4     AGUILAR COTA MARIA E/AU
E12           1     AGUILAR CRESPILLO C/AU

=> s e2-e6
              1 "AGUILAR CORDOBA E"/AU
              1 "AGUILAR CORDOVA C E"/AU
             57 "AGUILAR CORDOVA E"/AU
              1 "AGUILAR CORDOVA ESTAURDO"/AU
             17 "AGUILAR CORDOVA ESTUARDO"/AU
L11          77 ("AGUILAR CORDOBA E"/AU OR "AGUILAR CORDOVA C E"/AU OR "AGUILAR
                CORDOVA E"/AU OR "AGUILAR CORDOVA ESTAURDO"/AU OR "AGUILAR CORDO
                VA ESTUARDO"/AU)

=> s l11 and (Tat or Rev)
          7253 TAT
```

L12 5 L11 AND (TAT OR REV)

=> d l12,cbib,1-5

L12 ANSWER 1 OF 5 MEDLINE on STN
1999310662. PubMed ID: 10381536. A clinical trial of retroviral-mediated transfer of a **rev**-responsive element decoy gene into CD34(+) cells from the bone marrow of human immunodeficiency virus-1-infected children. Kohn D B; Bauer G; Rice C R; Rothschild J C; Carbonaro D A; Valdez P; Hao Q l; Zhou C; Bahner I; Kearns K; Brody K; Fox S; Haden E; Wilson K; Salata C; Dolan C; Wetter C; **Aguilar-Cordova E**; Church J. (Division of Research Immunology/Bone Marrow Transplantation, Childrens Hospital Los Angeles, CA 90027, USA.. dkohn@chla.usc.edu) . Blood, (1999 Jul 1) Vol. 94, No. 1, pp. 368-71. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

L12 ANSWER 2 OF 5 MEDLINE on STN
1998024131. PubMed ID: 9356449. TAK, an HIV **Tat**-associated kinase, is a member of the cyclin-dependent family of protein kinases and is induced by activation of peripheral blood lymphocytes and differentiation of promonocytic cell lines. Yang X; Gold M O; Tang D N; Lewis D E; **Aguilar-Cordova E**; Rice A P; Herrmann C H. (Division of Molecular Virology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA.) Proceedings of the National Academy of Sciences of the United States of America, (1997 Nov 11) Vol. 94, No. 23, pp. 12331-6. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

L12 ANSWER 3 OF 5 MEDLINE on STN
97288989. PubMed ID: 9143912. Protection of primary human T cells from HIV infection by Trev: a transdominant fusion gene. Chinen J; **Aguilar-Cordova E**; Ng-Tang D; Lewis D E; Belmont J W. (Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030, USA.) Human gene therapy, (1997 May 1) Vol. 8, No. 7, pp. 861-8. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

L12 ANSWER 4 OF 5 MEDLINE on STN
95339003. PubMed ID: 7614248. Inhibition of HIV-1 by a double transdominant fusion gene. **Aguilar-Cordova E**; Chinen J; Donehower L A; Harper J W; Rice A P; Butel J S; Belmont J W. (Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030, USA.) Gene therapy, (1995 May) Vol. 2, No. 3, pp. 181-6. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

L12 ANSWER 5 OF 5 MEDLINE on STN
94289068. PubMed ID: 8018390. A sensitive reporter cell line for HIV-1 **tat** activity, HIV-1 inhibitors, and T cell activation effects. **Aguilar-Cordova E**; Chinen J; Donehower L; Lewis D E; Belmont J W. (Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030.) AIDS research and human retroviruses, (1994 Mar) Vol. 10, No. 3, pp. 295-301. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

=> d l12,cbib,ab,1-5

L12 ANSWER 1 OF 5 MEDLINE on STN
1999310662. PubMed ID: 10381536. A clinical trial of retroviral-mediated transfer of a **rev**-responsive element decoy gene into CD34(+) cells from the bone marrow of human immunodeficiency virus-1-infected children. Kohn D B; Bauer G; Rice C R; Rothschild J C; Carbonaro D A; Valdez P; Hao Q l; Zhou C; Bahner I; Kearns K; Brody K; Fox S; Haden E; Wilson K; Salata C; Dolan C; Wetter C; **Aguilar-Cordova E**; Church J. (Division of Research Immunology/Bone Marrow Transplantation, Childrens Hospital Los Angeles, CA 90027, USA.. dkohn@chla.usc.edu) . Blood, (1999 Jul 1) Vol. 94, No. 1, pp. 368-71. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Genetic modification of hematopoietic stem cells with genes that inhibit replication of human immunodeficiency virus-1 (HIV-1) could lead to development of T lymphocytes and monocytic cells resistant to HIV-1 infection after transplantation. We performed a clinical trial to evaluate the safety and feasibility of this procedure, using bone marrow from four HIV-1-infected pediatric subjects (ages 8 to 17 years). We obtained bone marrow, isolated CD34(+) cells, performed in vitro transduction with a retroviral vector carrying a **rev**-responsive element (RRE) decoy gene, and reinfused the cells into these subjects with no evidence of adverse effects. The levels of gene-containing leukocytes in peripheral blood samples in the 1 year after gene transfer/cell infusion have been extremely low. These observations support the potential of performing gene therapy for HIV-1 using hematopoietic cells, but emphasize the need for improved gene transfer techniques.

1998024131. PubMed ID: 9356449. TAK, an HIV **Tat**-associated kinase, is a member of the cyclin-dependent family of protein kinases and is induced by activation of peripheral blood lymphocytes and differentiation of promonocytic cell lines. Yang X; Gold M O; Tang D N; Lewis D E; **Aguilar-Cordova E**; Rice A P; Herrmann C H. (Division of Molecular Virology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA.) Proceedings of the National Academy of Sciences of the United States of America, (1997 Nov 11) Vol. 94, No. 23, pp. 12331-6. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB We have previously identified a cellular protein kinase activity termed TAK that specifically associates with the HIV types 1 and 2 **Tat** proteins. TAK hyperphosphorylates the carboxyl-terminal domain of the large subunit of RNA polymerase II in vitro in a manner believed to activate transcription [Herrmann, C. H. & Rice, A. P. (1995) J. Virol. 69, 1612-1620]. We show here that the catalytic subunit of TAK is a known human kinase previously named PITALRE, which is a member of the cyclin-dependent family of proteins. We also show that TAK activity is elevated upon activation of peripheral blood mononuclear cells and peripheral blood lymphocytes and upon differentiation of U1 and U937 promonocytic cell lines to macrophages. Therefore, in HIV-infected individuals TAK may be induced in T cells following activation and in macrophages following differentiation, thus contributing to high levels of viral transcription and the escape from latency of transcriptionally silent proviruses.

L12 ANSWER 3 OF 5 MEDLINE on STN

97288989. PubMed ID: 9143912. Protection of primary human T cells from HIV infection by Trev: a transdominant fusion gene. Chinen J; **Aguilar-Cordova E**; Ng-Tang D; Lewis D E; Belmont J W. (Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030, USA.) Human gene therapy, (1997 May 1) Vol. 8, No. 7, pp. 861-8. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB Gene therapy is one of several approaches that are being tested in the search for an effective anti-human immunodeficiency virus (HIV) treatment. In this strategy, a "protective" gene would be introduced into target cells, rendering them relatively resistance to the virus-induced cytopathicity. **Tat** and **Rev** are viral proteins essential for HIV gene expression. **Tat** increases viral gene transcription and **Rev** is responsible for the nuclear export of mRNA encoding structural viral proteins. A fusion protein (Trev) was constructed, joining **Tat** and **Rev** transdominant mutant gene sequences. Previously, we showed that Trev inhibits both **Tat** and **Rev** activities in Jurkat T cells. To determine whether Trev could inhibit HIV replication in primary cells, we transferred the trev gene to peripheral blood lymphocytes and challenged them with different HIV strains. Levels of HIV p24 antigen (Ag) were reduced 4- to 15-fold in cultures of Trev-CD4+ T cells infected with two HIV primary clinical isolates and were not detectable in cultures infected with HIV strains NL4-3 and SF2. In contrast, cultures of nontransduced CD4+ T cells infected with the same viruses had levels of HIV p24 Ag up to 10 ng/ml. Trev-transduced CD4+ T cells demonstrated increased survival following HIV challenge for the length of the experiments (30 days). We did not observe rapid emergence of Trev-resistant HIV in our cultures. Following HIV challenge, cell-associated Trev protein was increased, supporting the hypothesis that cells surviving Trev expression provided a cell survival advantage. This work showed that Trev was able to inhibit HIV replication in primary CD4+ T cells, and, therefore the trev gene could be a candidate for gene therapy against HIV.

L12 ANSWER 4 OF 5 MEDLINE on STN

95339003. PubMed ID: 7614248. Inhibition of HIV-1 by a double transdominant fusion gene. **Aguilar-Cordova E**; Chinen J; Donehower L A; Harper J W; Rice A P; Butel J S; Belmont J W. (Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030, USA.) Gene therapy, (1995 May) Vol. 2, No. 3, pp. 181-6. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A double transdominant fusion gene (trev) designed to inhibit two essential HIV functions simultaneously was constructed by linking **tat** and **rev** transdominant mutants. Trev independently inhibited both **Tat** and **Rev** functions, localized within the nucleus and cells transfected with trev showed a stable inhibition of HIV-1-mediated cytopathicity. A retroviral vector of trev was made and shown also to confer protection from HIV cytopathic effects. Simultaneous inhibition of two essential viral genes presents significant advantages for potential gene therapy treatment of HIV infection over conventional single effect molecules.

L12 ANSWER 5 OF 5 MEDLINE on STN

94289068. PubMed ID: 8018390. A sensitive reporter cell line for HIV-1 **tat** activity, HIV-1 inhibitors, and T cell activation effects. **Aguilar-Cordova E**; Chinen J; Donehower L; Lewis D E; Belmont J W. (Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030.) AIDS research and human retroviruses, (1994 Mar) Vol. 10,

United States. Language: English.
AB The production and characterization of a cell line for quantitative HIV-1 **Tat** function and T cell activation assays is described. 1G5 is a clonal cell line derived from Jurkat T cells stably transfected with a luciferase gene driven by an HIV-1 long terminal repeat (HIV-LTR). The 1G5 clone was selected for low basal luciferase activity, susceptibility to HIV infection, and high responsiveness to **Tat** and T cell activation signals. A 10 to 1000-fold increase in luciferase activity after transfection or infection with **tat**-expressing vectors or HIV was observed. Equivalent levels of expression were detected after stimulation with T cell mitogens. The characteristics of 1G5 make it a valuable reagent for studies of HIV infection, HIV regulatory agents, and other T cell or HIV-activating factors, and for screening potential anti-HIV therapeutic agents.

=> d his

(FILE 'HOME' ENTERED AT 08:23:09 ON 27 JUN 2006)

FILE 'USPATFULL' ENTERED AT 08:23:19 ON 27 JUN 2006
E AGUILAR-CORDOVA C E/IN
E CORDOVA C E A/IN
E AGUILAR CORDOVA C E/IN
L1 8 S E4-E7
E BELMONT JOHN W/IN
L2 3 S E3
L3 2 S L2 NOT L1
E HARPER J WADE/IN
L4 1 S E3
L5 0 S L4 NOT L1

FILE 'WPIDS' ENTERED AT 08:26:29 ON 27 JUN 2006
E AGUILAR CORDOVA C E/IN
L6 9 S E2-E5
E BELMONT J W/IN
L7 2 S E3
L8 1 S L7 NOT L6
E HARPER J W/IN
L9 10 S E3
L10 9 S L9 NOT L6

FILE 'USPATFULL' ENTERED AT 08:27:55 ON 27 JUN 2006
E AGUILAR CORDOVA C E/AU

FILE 'MEDLINE' ENTERED AT 08:28:23 ON 27 JUN 2006
E AGUILAR CORDOVA C E/AU
L11 77 S E2-E6
L12 5 S L11 AND (TAT OR REV)

=> e belmont j w/au
E1 16 BELMONT J M/AU
E2 4 BELMONT J R/AU
E3 69 --> BELMONT J W/AU
E4 10 BELMONT JOHN/AU
E5 6 BELMONT JOHN M/AU
E6 25 BELMONT JOHN W/AU
E7 4 BELMONT JONATHAN/AU
E8 2 BELMONT JONATHAN B/AU
E9 30 BELMONT L/AU
E10 7 BELMONT L D/AU
E11 1 BELMONT LAGUNA F/AU
E12 1 BELMONT LAGUNA FRANCISCO/AU

=> s e3 or e4 or e6
69 "BELMONT J W"/AU
10 "BELMONT JOHN"/AU
25 "BELMONT JOHN W"/AU
L13 104 "BELMONT J W"/AU OR "BELMONT JOHN"/AU OR "BELMONT JOHN W"/AU

=> s l13 not l11
L14 99 L13 NOT L11

=> s l14 and (Tat or Rev)
7253 TAT
5692 REV
L15 0 L14 AND (TAT OR REV)

=> e harper j w/au
E1 6 HARPER J T/AU
E2 10 HARPER J V/AU
E3 105 --> HARPER J W/AU
E4 1 HARPER J W 2ND/AU

```
E6              6     HARPER J Y JR/AU
E7              2     HARPER JACQUELINE/AU
E8              3     HARPER JACQUIE L/AU
E9              4     HARPER JAMES/AU
E10             5     HARPER JAMES A/AU
E11             1     HARPER JAMES D/AU
E12             1     HARPER JAMES J 3RD/AU

=> s e3-e5
            105 "HARPER J W"/AU
              1 "HARPER J W 2ND"/AU
             42 "HARPER J WADE"/AU
L16         148 ("HARPER J W"/AU OR "HARPER J W 2ND"/AU OR "HARPER J WADE"/AU)

=> s l16 not l11
L17         147 L16 NOT L11

=> s l17 and (Tat or Rev)
           7253 TAT
           5692 REV
L18           1 L17 AND (TAT OR REV)

=> d l18,cbib

L18  ANSWER 1 OF 1     MEDLINE on STN
91329382.    PubMed ID: 1868081.   Refolded HIV-1 tat protein protects both
     bulge and loop nucleotides in TAR RNA from ribonucleolytic cleavage.
     Harper J W; Logsdon N J. (Verna and Marrs McLean Department of
     Biochemistry, Baylor College of Medicine, Houston, Texas 77030. )
     Biochemistry, (1991 Aug 13) Vol. 30, No. 32, pp. 8060-6.  Journal code:
     0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.


=> file uspatful
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       4.85      38.12

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=> s (Tat and Rev)
          28027 TAT
          95430 REV
L19       13995 (TAT AND REV)

=> s l19 and transdominant
            525 TRANSDOMINANT
L20         296 L19 AND TRANSDOMINANT

=> s l20 and (Tat/clm and Rev/clm)
           1477 TAT/CLM
            626 REV/CLM
L21          36 L20 AND (TAT/CLM AND REV/CLM)

=> s l21 and ay<1996
        2275616 AY<1996
L22           6 L21 AND AY<1996

=> d l22,cbib,1-6

L22  ANSWER 1 OF 6  USPATFULL on STN
2002:112895 RIBOZYMES TARGETING THE RETROVIRAL PACKAGING SEQUENCE EXPRESSION
    CONSTRUCTS AND RECOMBINANT RETROVIRUSES CONTAINING SUCH CONSTRUCTS.
    SYMONDS, GEOFFREY P., ROSE BAY, AUSTRALIA
    SUN, LUN-QUAN, RYDE, AUSTRALIA
    US 2002058636 A1 20020516
    APPLICATION: US 1995-375291  A1  19950118 (8)
    PRIORITY: WO 1995-IB50  19950105
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
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2002:84898 PROTEIN-POLYCATION CONJUGATES.
BIRNSTIEL, MAX L., WIEN, AUSTRIA
COTTEN, MATTHEW, WIEN, AUSTRIA
WAGNER, ERNST, LANGENZERSDORF, AUSTRIA
US 2002044937 A1 20020418
APPLICATION: US 1995-380200 A1 19950130 (8)
PRIORITY: WO 1991-EP875 19910510
AT 1990-1110 19900518
DE 1991-4110410 19910329
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L22 ANSWER 3 OF 6 USPATFULL on STN
1999:141665 Composition of trans-dominant variants of viral proteins for obtaining an antiviral effect.
Mehtali, Majid, Illkirch-Graffenstaden, France
Guss, Tania, Dossenheim-sur-Zinsel, France
Transgene S.A., Strasbourg, France (non-U.S. corporation)
US 5981258 19991109
WO 9516780 19950622
APPLICATION: US 1995-505210 19950814 (8)
WO 1994-FR1457 19941213 19950814 PCT 371 date 19950814 PCT 102(e) date
PRIORITY: FR 1993-14914 19931213
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L22 ANSWER 4 OF 6 USPATFULL on STN
1998:134622 Method for destroying a diseased human cell.
Gruber, Harry E., P.O. Box 675272, Rancho Santa Fe, CA, United States 92067
Jolly, Douglas J., 277 Hillcrest Dr., Leucadia, CA, United States 92024
Respess, James G., 4966 Lamont St., San Diego, CA, United States 92109
Laikind, Paul K., 3370 Goldfinch St., San Diego, CA, United States 92103
US 5830458 19981103
APPLICATION: US 1995-487776 19950607 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L22 ANSWER 5 OF 6 USPATFULL on STN
1998:33578 Introduction of HIV-protective genes into cells by particle-mediated gene transfer.
Nabel, Gary J., 3390 Andover Rd., Ann Arbor, MI, United States 48105
Woffendin, Clive, 3509 Burbank Dr., Ann Arbor, MI, United States 48105
Yang, Nin-Sun, 7802 Oxtrail Way, Verona, WI, United States 53593
Sheehy, Michael J., 629 Piper Dr., Madison, WI, United States 53711
US 5733543 19980331
APPLICATION: US 1994-235277 19940429 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L22 ANSWER 6 OF 6 USPATFULL on STN
97:63912 Viral vectors.
Wong-Staal, Flossie, San Diego, CA, United States
Mamounas, Michael, Solana Beach, CA, United States
Poeschla, Eric M., San Diego, CA, United States
Kraus, Gunter, La Jolla, CA, United States
Leavitt, Mark, La Jolla, CA, United States
The Regents of the University of California, Oakland, CA, United States (U.S. corporation)
US 5650309 19970722
APPLICATION: US 1995-442061 19950516 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l22,cbib,clm,1-6

L22 ANSWER 1 OF 6 USPATFULL on STN
2002:112895 RIBOZYMES TARGETING THE RETROVIRAL PACKAGING SEQUENCE EXPRESSION CONSTRUCTS AND RECOMBINANT RETROVIRUSES CONTAINING SUCH CONSTRUCTS.
SYMONDS, GEOFFREY P., ROSE BAY, AUSTRALIA
SUN, LUN-QUAN, RYDE, AUSTRALIA
US 2002058636 A1 20020516
APPLICATION: US 1995-375291 A1 19950118 (8)
PRIORITY: WO 1995-IB50 19950105
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A synthetic non-naturally occurring oligonucleotide compound which comprises nucleotides whose sequence defines a conserved catalytic region and nucleotides whose sequence is capable of hybridizing with a predetermined target sequence within a packaging sequence of an RNA

2. The compound of claim 1, wherein the viral packaging sequence of is a retrovirus packaging sequence.

3. The compound of claim 1, wherein the packaging sequence is the HIV-1 Psi packaging sequence.

4. The compound of claim 1, wherein the RNA virus is a Feline Leukemia Virus.

5. The compound of claim 1, wherein the RNA virus is a Feline Immunodeficiency Virus.

6. The compound of claim 1 having the structure: ##STR4## wherein each X represents a nucleotide which is the same or different and may be modified or substituted in its sugar, phosphate or base; wherein each of A, C, U, and G represents a ribonucleotide which may be unmodified or modified or substituted in its sugar, phosphate or base; wherein 3'--AAG . . . AGUCX--5' defines the conserved catalytic region; wherein each of $(X)_{nA}$ and $(X)_n$, defines the nucleotides whose sequence is capable of hybridizing with the predetermined target sequence within the packaging sequence of the RNA virus; wherein each * represents base pairing between the nucleotides located on either side thereof; wherein each solid line represents a chemical linkage providing covalent bonds between the nucleotides located on either side thereof; wherein each of the dashed lines independently represents either a chemical linkage providing covalent bonds between the nucleotides located on either side thereof or the absence of any such chemical linkage; wherein a represents an integer which defines a number of nucleotides with the proviso that a may be 0 or 1 and if 0, the A located 5' of $(X)_a$ is bonded to the X located 3' of $(X)_a$; wherein each of m and m' represents an integer which is greater than or equal to 1; wherein $(X)_b$ represents an oligonucleotide and b represents an integer which is greater than or equal to 2.

7. The compound of claim 1 having the structure: ##STR5## wherein each X is the same or different and represents a ribonucleotide or a deoxyribonucleotide which may be modified or substituted in its sugar, phosphate or base; wherein each of A, C, U, and G represents a ribonucleotide which may be unmodified or modified or substituted in its sugar, phosphate or base; wherein 3'--AAG . . . AGUCX--5 defines the conserved catalytic region; wherein each of $(X)_{nA}$ and $(X)_n$, defines the nucleotides whose sequence is capable of hybridizing with the predetermined target sequence within the packaging sequence of an RNA virus; wherein each solid line represents a chemical linkage providing covalent bonds between the nucleotides located on either side thereof; wherein m represents an integer from 2 to 20; and wherein none of the nucleotides $(X)_m$ are Watson-Crick base paired to any other nucleotide within the compound.

8. The compound of claim 1 having the structure: ##STR6## wherein each X is the same or different and represents a ribonucleotide or a deoxyribonucleotide which may be modified or substituted in its sugar, phosphate or base; wherein each of A, C, U, and G represents a ribonucleotide which may be unmodified or modified or substituted in its sugar, phosphate or base; wherein 3'$(X)_{P4}$ . . . $(X)_{P1}$--5' defines the conserved catalytic region; wherein each of $(X)_{F4}$ and $(X)_{F3}$ defines the nucleotides whose sequence is capable of hybridizing with the predetermined target sequence within the packaging sequence of an RNA virus; wherein each solid line represents a chemical linkage providing covalent bonds between the nucleotides located on either side thereof; wherein F3 represents an integer which defines the number of nucleotides in the oligonucleotide with the proviso that F3 is greater than or equal to 3; wherein F4 represents an integer which defines the number of nucleotides in the oligonucleotide with the proviso that F4 is from 3 to 5; wherein each of $(X)_{P1}$ and $(X)_{P4}$ represents an oligonucleotide having a predetermined sequence such that $(X)_{P4}$ base-pairs with 3-6 bases of $(X)P_1$; wherein P1 represents an integer which defines the number of nucleotides in the oligonucleotide with the proviso that P1 is from 3 to 6 and the sum of P1 and F4 equals 9; wherein each of $(X)_{P2}$ and $(X)_{P3}$ represents an oligonucleotide having a predetermined sequence such that $(X)_{P2}$ base-pairs with at least 3 bases of $(X)_{P3}$; wherein each * represents base pairing between the nucleotides located on either side thereof; wherein each solid line represents a chemical linkage providing covalent bonds between the nucleotides located on either side thereof; wherein each of the dashed lines independently represents either a chemical linkage providing covalent bonds between the nucleotides located on either side thereof or the absence of any such chemical linkage; and wherein $(X)_{L2}$ represents an oligonucleotide which may be present or absent with the proviso that L2 represents an integer

9. The compound of claim 1, wherein the nucleotides whose sequences define a conserved catalytic region are from the hepatitis delta virus conserved region.

10. The compound of claim 1, wherein the nucleotides whose sequences define a conserved catalytic region contain the sequence NCCA at its 3' terminus.

11. A synthetic non-naturally occurring oligonucleotide compound which comprises two or more domains which may be the same or different wherein each domain comprises nucleotides whose sequence defines a conserved catalytic region and nucleotides whose sequence is capable of hybridizing with a predetermined target sequence within a packaging sequence of an RNA virus.

12. The compound of claim 1 and further comprising a covalently linked antisense nucleic acid compound capable of hybridizing with a predetermined sequence, which may be the same or different, within a packaging sequence of the RNA virus.

13. The compound of claim 1, wherein the nucleotides are capable of hybridizing with the 243, 274, 366 or 553 target sequence in the MOMLV, and site 749 in the HIV Psi packaging site.

14. A compound comprising the compound of claim 1, and further comprising at least one additional synthetic non-naturally occuring oligonucleotide compound with or without an antisense molecule covalently linked, and targeted to a different gene of the RNA virus genome.

15. The compound of claim 14, wherein the RNA virus is HIV and the different region of the HIV genome is selected from the group consisting of long terminal repeat, 5' untranslated region, splice donor-acceptor sites, primer binding sites, 3' untranslated region, gag, pol, protease, integrase, env, **tat**, **rev**, nef, vif, vpr, vpu, vpx, or tev region.

16. The compound of claim 15, wherein the nucleotides are capable of hybridizing with the 243, 274, 366 or 553 target sites or combination thereof in the MOMLV and site 749 in the HIV Psi packaging site and the nucleotides of the additional compound are capable of hybridizing with the 5792, 5849, 5886, or 6042 target sites or combination thereof in the HIV **tat** region.

17. A composition which comprises the compound of claims 1 or 14 in association with a pharmaceutically, veterinarially, or agriculturally acceptable carrier or excipient.

18. A composition which comprises the compound of claim 1, with or without antisense, and further comprises a TAR decoy, polyTAR or a RRE decoy.

19. A method for producing the compound of claim 1 which comprises the steps of: (a) ligating into a transfer vector comprised of DNA, RNA or a combination thereof a nucleotide sequence corresponding to the compound; (b) transcribing the nucleotide sequence of step (a) with an RNA polymerase; and (c) recovering the compound.

20. A transfer vector comprised of RNA or DNA or a combination thereof containing a nucleotide sequence which on transcription gives rise to the compound of claim 1.

21. The transfer vector of claim 20, wherein the transfer vector comprises the HIV long terminal repeat, an adenovirus associated transfer vector, an SV40 promoter, Mo-MLV, or an amphotropic retrovirus vector.

22. The transfer vector of claim 20 further comprising a sequence directing the oligonucleotide compound to a particular organ or cell in vivo or a particular region within the cell.

23. A composition which comprises the transfer vector of claim 20 in association with a pharmaceutically, veterinarially or agriculturally acceptable carrier or excipient.

24. A prokaryotic or eukaryotic cell comprising a nucleotide sequence which is, or on transcription give s rise to the compound of claim 1.

25. The cell of claim 24, wherein the cell is a eukaryotic cell.

26. The eukaryotic cell of claim 25, wherein the cell is an animal cell.

stem cell which gives rise to progenitor cells, more mature, and fully mature cells of all the hematopoietic cell lineages.

28. The eukaryotic cell of claim 25, wherein the cell is a progenitor cell which gives rise mature cells of all the hematopoietic cell lineages.

29. The eukaryotic cell of claim 25, wherein the cell is a committed progenitor cell which gives rise to a specific hematopoietic lineage.

30. The eukaryotic cell of claim 25, wherein the cell is a T lymphocyte progenitor cell.

31. The eukaryotic cell of claim 25, wherein the cell is an immature T lymphocyte.

32. The eukaryotic cell of claim 25, wherein the cell is a mature T lymphocyte.

33. The eukaryotic cell of claim 25, wherein the cell is a myeloid progenitor cell.

34. The eukaryotic cell of claim 25, wherein the cell is a monocyte/macrophage cell.

35. The use of the compound of claims 1 to protect hematopoietic stem cells, progenitor cells, committed progenitor cells, T lymphocyte progenitor cells, immature T lymphocytes, mature T lymphocytes, myeloid progenitor cells, or monocyte/macrophage cells.

36. A method to suppress HIV in an AIDS patient which comprises the introduction of the transfer vector of claim 20 into hematopoietic cells thereby rendering the cells resistant to HIV so as to thereby suppress HIV in an AIDS patient.

37. The method of claim 36, wherein the introduction is ex vivo and the cells are autologous or heterologous cells.

38. The method of claim 36, wherein the introduction is ex vivo and the cells are transplanted without myeloablation.

39. The method of claim 36, wherein the introduction is ex vivo and the cells are transplanted with myeloablation.

40. The method of claim 37, wherein the cells are also treated with an additional agent to inhibit or eliminate HIV-1 replication.

41. The method of claim 40, wherein the additional agent is a neutralizing antibody such as IgGlbl2; a nucleoside analogues such as zidovudine (AZT), ddI, ddC, d4t; a non-nucleoside reverse transcriptase inhibitors such as nevirapine, delavirdine, lamivudine (3-TC), loviride; or a protease inhibitors such as saquinavir.

42. A method for protecting an individual from HIV infection which comprises incorporation of the transfer vector of claim 20 into the individual's cells thereby protecting that individual from the effects of high levels of the virus.

L22 ANSWER 2 OF 6 USPATFULL on STN
2002:84898 PROTEIN-POLYCATION CONJUGATES.
BIRNSTIEL, MAX L., WIEN, AUSTRIA
COTTEN, MATTHEW, WIEN, AUSTRIA
WAGNER, ERNST, LANGENZERSDORF, AUSTRIA
US 2002044937 A1 20020418
APPLICATION: US 1995-380200 A1 19950130 (8)
PRIORITY: WO 1991-EP875 19910510
AT 1990-1110 19900518
DE 1991-4110410 19910329
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. The protein-polycation conjugates which are capable of forming, with nucleic acids or nucleic acid analogues, soluble complexes which are absorbed into human or animal cells, characterised in that the protein component of the conjugates is a protein capable of binding to a cell surface protein expressed by cells of the T-cell lineage, so that the complexes formed are taken up into cells which express the T-cell surface protein.

2. Conjugates according to claim 1,characterised in that their protein component is a preferably monoclonal antibody or a fragment thereof,

3. Conjugates according to claim 1 or 2, characterised in that they contain a protein capable of binding to CD4.

4. Conjugates according to claim 3 characterised in that they contain a monoclonal anti-CD4 antibody or the fragment thereof which contains a gp120 binding epitope.

5. Conjugates according to claim 3, characterised in that they contain as protein HIV-1 gp120 or a homologous protein of related retroviruses or a fragment thereof which binds to CD4.

6. Conjugates according to claim 1 or 2, characterised in that they contain a protein which binds to a tumour marker expressed on T-cells.

7. Conjugates according to claim 6, characterised in that they contain a protein which binds to CD7.

8. Conjugates according to one of claims 2, 4, 6 or 7, characterised in that they contain an antibody in a form which is directly coupled to the polycation.

9. Conjugates according to one of claims 2, 4, 6 or 7, characterised in that they contain an antibody in a form bound by means of a protein A coupled to polycation.

10. Protein A-polycation conjugates for preparing antibody conjugates according to claim 9.

11. Conjugates according to claim 1, characterised in that the polycation is an optionally modified protamine.

12. Conjugates according to claim 1, characterised in that the polycation is an optionally modified histone.

13. Conjugates according to claim 1, characterised in that the polycation is a synthetic homologous or heterologous polypeptide.

14. Conjugates according to claim 13, characterised in that the polypeptide is polylysine.

15. Conjugates according to one of claims 11 to 14, characterised in that the polycation has about 20 to 500 positive charges.

16. Conjugates according to one of claims 11 to 15, characterised in that the molar ratio of T-cell binding protein to polycation is about 10:1 to 1:10.

17. New protein-polycation/nucleic acid complexes which are absorbed into human or animal cells, characterised in that the protein component of the conjugates is a protein capable of binding to a cell surface protein expressed by cells of the T-cell lineage, so that the complexes formed are taken up in cells which express the T-cell surface protein.

18. Complexes according to claim 17, characterised in that they contain as the conjugate component one of the conjugates defined in claims 1 to 9 or 11 to 16.

19. Complexes according to one of claims 17 or 18, characterised in that they additionally contain a non-covalently bound polycation, which may optionally be identical to the polycation of the conjugate, so that the internalisation and/or expression of the nucleic acid achieved by the conjugate is increased.

20. Complexes according to one of claims 17 to 19, characterised in that they contain a virus inhibiting nucleic acid.

21. Complexes according to claim 20, characterised in that they contain a nucleic acid which inhibits replication and expression of the HIV-1 virus or related retroviruses.

22. Complexes according to claim 21, characterised in that the inhibiting nucleic acid is complementary to sequences of the HIV-1 genome.

23. Complexes according to claim 22, characterised in that the nucleic acid is complementary to sequences of the **tat** gene.

24. Complexes according to claim 22, characterised in that the nucleic acid is complementary to sequences of the **rev** gene.

25. Complexes according to claim 22, characterised in that the nucleic

26. Complexes according to claim 22, characterised in that the nucleic acid is complementary to LTR-sequences.

27. Complexes according to claim 22, characterised in that the nucleic acid is complementary to the tar sequence.

28. Complexes according to one of claims 20 to 27, characterised in that they contain an inhibiting nucleic acid in the form of a ribozyme, optionally together with a carrier RNA, or the gene coding therefor.

29. Complexes according to claim 28, characterised in that they contain a nucleic acid in the form of a genetic unit consisting of a tRNA-gene as carrier gene and a ribozyme gene arranged within this gene.

30. Complexes according to one of claims 20 to 27, characterised in that they contain an inhibiting nucleic acid in the form of an optionally modified antisense oligonucleotide, optionally together with a carrier nucleic acid, or in the case of an RNA-oligonucleotide, the gene coding therefor.

31. Complexes according to claim 20, characterised in that they contain a nucleic acid coding for a virus protein which contains a trans-dominant mutation.

32. Complexes according to one of claims 17 to 19, characterised in that they contain an oncogene-inhibiting nucleic acid.

33. Complexes according to claim 32, characterised in that they contain an oncogene-inhibiting nucleic acid in the form of a ribozyme, optionally together with a carrier RNA or the gene coding therefor.

34. Complexes according to claim 33, characterised in that they contain an oncogene-inhibiting nucleic acid in the form of a ribozyme, optionally together with a carrier RNA, or the gene coding therefor.

35. Complexes according to one of claims of 17 to 19, characterised in that they contain as nucleic acid a therapeutically or gene therapeutically active gene or gene section.

36. Process for introducing nucleic acid or acids into cells which express a T-cell surface protein by forming one of the complexes defined in claims 17 to 35, which is preferably soluble under physiological conditions, from one of the protein-polycation conjugates defined in claims 1 to 9 or 11 to 16 and nucleic acid or acids, optionally in the presence of non-covalently bound polycation, and bringing cells which express the T-cell surface protein, especially T-cells, into contact with this complex, optionally under conditions under which the breakdown of nucleic acid in the cell is inhibited.

37. Process according to claim 36, in which a complex is formed from a protein A-polycation conjugate, consisting of protein A and one of the polycations defined in claims 11 to 15 and one of the nucleic acids defined in claims 20 to 35, and the complex is brought into contact, in the presence of an antibody directed against a T-cell surface protein, with cells which express this surface protein, the antibody being bound to the conjugate component of the complex.

38. Pharmaceutical preparation containing as active component one or more therapeutically or gene therapeutically active nucleic acids in the form of one of the complexes defined in claims 17 to 35.

L22 ANSWER 3 OF 6 USPATFULL on STN
1999:141665 Composition of trans-dominant variants of viral proteins for obtaining an antiviral effect.
Mehtali, Majid, Illkirch-Graffenstaden, France
Guss, Tania, Dossenheim-sur-Zinsel, France
Transgene S.A., Strasbourg, France (non-U.S. corporation)
US 5981258 19991109
WO 9516780 19950622
APPLICATION: US 1995-505210 19950814 (8)
WO 1994-FR1457 19941213 19950814 PCT 371 date 19950814 PCT 102(e) date
PRIORITY: FR 1993-14914 19931213
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A composition comprising at least: (a) a first trans-dominant variant of the **TAT** protein of human immunodeficiency virus (HIV); said trans-dominant variant of the **TAT** protein having the sequence of SEQ ID NO: 1 which comprises at least one of the following modifications: (i) the phenylalanine residue at position +38 is replaced by an aspartic

an alanine residue; (iii) the lysine residue at position +41 is replaced by a glutamic acid residue; (iv) the isoleucine residue at position +45 is replaced by a serine residue; and (v) the tyrosine residue at position +47 is replaced by an arginine residue; and (b) a second trans-dominant variant of the **REV** protein of HIV; said trans-dominant variant of the **REV** protein having the sequence of SEQ ID NO: 2 which comprises at least the following modifications: (i) the glutamine residue at position +74 is replaced by a glycine residue; and (ii) the leucine residue at position +75 is replaced by a serine residue; wherein said composition inhibits viral replication.

2. The composition of claim 1, wherein the tram-dominant variant of the **REV** protein has the sequence of SEQ ID NO: 2 wherein the glutamine residue at position +74 is replaced by a glycine residue, the leucine residue at position +75 is replaced by a serine residue, the leucine residue at position +78 is replaced by a glutamic acid residue the glutamic acid residue at position +79 is replaced by a phenylalanine residue and the leucine residue at position +81 is replaced by an aspartic acid residue.

3. A recombinant vector comprising: (a) a first DNA sequence coding for a first trans-dominant variant of the **TAT** protein of HIV, placed under the control of the elements needed for its expression; said trans-dominant variant of the **TAT** protein having the sequence of SEQ ID NO: 1 which comprises at least one of the following modifications: (i) the phenylalanine residue at position +38 is replaced by an aspartic acid residue; (ii) the threonine residue at position +40 is replaced by an alanine residue; (iii) the lysine residue at position +41 is replaced by a glutamic acid residue; (iv) the isoleucine residue at position +45 is replaced by a serine residue; and (v) the tyrosine residue at position +47 is replaced by an arginine residue; and (b) a second DNA sequence coding for a second trans-dominant variant of the **REV** protein of HIV, placed under the control of the elements needed for its expression; said trans-dominant variant of the **REV** protein having the sequence of SEQ ID NO: 2 which comprises at least the following modifications: (i) the glutamine residue at position +74 is replaced by a glycine residue; and (ii) the leucine residue at position +75 is replaced by a serine residue; wherein said vector inhibits viral replication.

4. The recombinant vector of claim 3, wherein said vector is a virus selected from the group consisting of retroviruses, adenoviruses and adeno-associated viruses.

5. The composition of claim 1, which further comprises a pharmaceutically acceptable carrier.

6. The recombinant vector of claim 3, wherein said trans-dominant variant of the **REV** protein has the sequence of SEQ ID NO: 2 wherein the glutamine residue at position +74 is replaced by a glycine residue, the leucine residue at position +75 is replaced by a serine residue, the leucine residue at position +78 is replaced by a glutamic acid residue, the glutamic acid residue at position +79 is replaced by a phenylalanine residue and the leucine residue at position +81 is replaced by an aspartic acid residue.

7. A eukaryotic cell into the genome of which there are inserted: (a) a first DNA sequence coding for a first trans-dominant variant of the **TAT** protein of HIV, placed under the control of the elements needed for its expression; said trans-dormant variant of the **TAT** protein having the sequence of SEQ ID NO: 1 which comprises at least one of the following modifications: (i) the phenylalanine residue at position +38 is replaced by an aspartic acid residue; (ii) the threonine residue at position +40 is replaced by an alanine residue; (iii) the lysine residue at position +41 is replaced by a glutamic acid residue; (iv) the isoleucine residue at position +45 is replaced by a serine residue; and (v) the tyrosine residue at position +47 is replaced by an arginine residue; and (b) a second DNA sequence coding for a second trans-dominant variant of the **REV** protein of HIV, placed under the control of the elements needed for its expression; said trans-dominant variant of the **REV** protein having the sequence of SEQ ID NO: 2 which comprises at least the following modifications: (i) the glutamine residue at position +74 is replaced by a glycine residue; and (ii) the leucine residue at position +75 is replaced by a serine residue; wherein said cell inhibits viral replication.

8. The eukaryotic cell of claim 7, wherein said trans-dominant variant of the **REV** protein has the sequence of SEQ ID NO: 2 wherein the glutamine residue at position +74 is replaced by a glycine residue, the leucine residue at position +75 is replaced by a serine residue, the leucine residue at position +78 is replaced by a glutamic acid residue, the glutamic acid residue at position +79 is replaced by a phenylalanine

aspartic acid residue.

9. A kit comprising: (a) a first trans-dominant variant of the **TAT** protein of human immunodeficiency virus (HIV); said trans-dominant variant of the **TAT** protein having the sequence of SEQ ID NO: 1 which comprises at least one of the following modifications: (i) the phenylalanine residue at position +38 is replaced by an aspartic acid residue; (ii) the threonine residue at position +40 is replaced by an alanine residue; (iii) the lysine residue at position +41 is replaced by a glutamic acid residue; (iv) the isoleucine residue at position +45 is replaced by a serine residue; and (v) the tyrosine residue at position +47 is replaced by an arginine residue; and (b) a second trans-dominant variant of the **REV** protein of HIV; said trans-dominant variant of the **REV** protein having the sequence of SEQ ID NO: 2 which comprises at least the following modifications: (i) the glutamine residue at position +74 is replaced by a glycine residue; and (ii) the leucine residue at position +75 is replaced by a serine residue; wherein the combination of said first and second trans-dominant variant inhibits viral replication.

10. The kit of claim 9, wherein said trans-dominant variant of the **REV** protein has the sequence of SEQ ID NO: 2, wherein the glutamine residue at position +74 is replaced by a glycine residue, the leucine residue at position +75 is replaced by a serine residue, the leucine residue at position +78 is replaced by a glutamic acid residue, the glutamic acid residue at position +79 is replaced by a phenylalanine residue and the leucine residue at position +81 is replaced by an aspartic acid residue.

L22 ANSWER 4 OF 6 USPATFULL on STN
1998:134622 Method for destroying a diseased human cell.
Gruber, Harry E., P.O. Box 675272, Rancho Santa Fe, CA, United States 92067
Jolly, Douglas J., 277 Hillcrest Dr., Leucadia, CA, United States 92024
Respess, James G., 4966 Lamont St., San Diego, CA, United States 92109
Laikind, Paul K., 3370 Goldfinch St., San Diego, CA, United States 92103
US 5830458 19981103
APPLICATION: US 1995-487776 19950607 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method for destroying a diseased human cell, said method comprising: a) infecting a human cell with a replication defective recombinant retrovirus comprising a recombinant gene operatively linked to a promoter, said gene encoding a protein which converts a purine-based or pyrimidine-based drug to a second compound that is toxic to said infected human cell; b) administering said purine-based or pyrimidine-based drug to said infected human cell when said cell is in a diseased state, said diseased state being a viral infection, a cancer, or graft versus host disease, said purine-based or pyrimidine-based drug reacting with said protein to form a therapeutic agent that is toxic to said diseased cell in an amount that is lethal to said cell, whereby said diseased cell is destroyed.

2. The method of claim 1, wherein said diseased state is a member of the group consisting of a viral infection and a cancerous disease.

3. The method of claim 1, wherein said gene is of non-mammalian origin.

4. The method of claim 1, wherein said recombinant retrovirus further comprises a second gene encoding a cell surface marker.

5. The method of claim 2, wherein said cancerous disease is melanoma, prostate cancer, cervical carcinoma, colon carcinoma, or hepatocarcinoma.

6. The method of claim 2, wherein said promoter is a promoter for GD2 antigen, prostate specific antigen, human papilloma virus, p53, or transferrin receptor.

7. The method of claim 2, wherein said diseased state is a viral infection.

8. The method of claim 3, wherein said gene is of viral, bacterial, fungal or protozoal origin.

9. The method of claim 7, wherein said promoter is a viral specific transcriptional promoter whose expression is stimulated by a virus-specific trans-activating protein.

10. The method of claim 7, wherein said viral infection is an HIV infection.

12. The method of claim 8, wherein the gene is of bacterial origin.

13. The method of claim 9, wherein a portion of said recombinant retrovirus is of HIV origin.

14. The method of claim 9, wherein said viral specific transcriptional promoter is responsive to **rev** or **tat**.

15. The method of claim 9, wherein said gene is of non-mammalian origin.

16. The method of claim 11, wherein the gene is a herpes simplex virus thymidine kinase gene.

17. The method of claim 11, wherein the purine-based or pyrimidine-based drug is acyclovir (ACV), AZT, ddC, FIAU, FIAC or DHPG.

18. The method of claim 12, wherein the gene encodes a guanine phosphoribosyl transferase (gpt).

19. The method of claim 14, wherein said recombinant retrovirus comprises said gene operatively controlled by a first promoter responsive to **rev** and a second promoter responsive to **tat**.

20. The method of claim 14, wherein said promoter in said recombinant retrovirus comprises multimers of a **tat**-responsive element.

21. The method of claim 14, wherein said promoter in said recombinant retrovirus comprises multimers of a **rev**-responsive element.

22. The method of claim 15, wherein said gene is of viral, bacterial, fungal or protozoal origin.

23. The method of claim 15, wherein said gene is of bacterial origin.

24. The method of claim 18, wherein said bacterial origin of said gpt is E. coli.

25. The method of claim 19, wherein said first promoter in said recombinant retrovirus comprises multimers of a **rev**-responsive element.

26. The method of claim 19, wherein said second promoter in said recombinant retrovirus comprises multimers of a **tat**-responsive element.

27. The method of claim 20, wherein said **tat**-responsive element is "TAR" of HIV.

28. The method of claim 21, wherein said **rev**-responsive element is "CRS/CAR" of HIV.

29. The method of claim 22, wherein said gene is of viral origin.

30. The method of claim 22, wherein said recombinant retrovirus further comprises a second gene encoding a cell surface marker.

31. The method of claim 23, wherein said gene encodes guanine phosphoribosyl transferase.

32. The method of claim 24, wherein said purine-based or pyrimidine-based drug is thioxanthine.

33. The method of claim 29, wherein said gene encodes a viral thymidine kinase.

34. The method of claim 31, wherein said bacterial origin of said gene is E. coli.

35. The method of claim 33, wherein said thymidine kinase is a herpes simplex virus thymidine kinase.

36. The method of claim 34, wherein said purine-based or pyrimidine-based drug is thioxanthine.

37. The method of claim 35, wherein the purine-based or pyrmidine-based drug is acyclovir (ACV), AZT, ddC, FIAU, FIAC or DHPG.

38. The method of claim 35, wherein said recombinant retrovirus further comprises a gene encoding a cell surface marker.

L22 ANSWER 5 OF 6 USPATFULL on STN
1998:33578 Introduction of HIV-protective genes into cells by particle-mediated

Nabel, Gary J., 3390 Andover Rd., Ann Arbor, MI, United States 48105
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Sheehy, Michael J., 629 Piper Dr., Madison, WI, United States 53711
US 5733543 19980331
APPLICATION: US 1994-235277 19940429 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A method for prolonging T cell survival in a HIV infected patient, comprising: (i) removing a plurality oft cells from a said patient; (ii) introducing, by particle mediated gene transfer, a gene encoding a product which inhibits HIV replication into said plurality of T cells; and (iii) reintroducing said plurality of T cells into said patient, wherein said gene encoding a product which inhibits HIV replication is **Rev** M10.

2. The method of claim 1, wherein said gene is under the operational control of a sequence of DNA such that the expression of said gene is stimulated by the expression of HIV.

3. The method of claim 1, wherein said gene is contained in a plasmid and is downstream from the TAR sequence such that expression of the gene is activated by **Tat**.

4. The method of claim 3, wherein said plasmid is pRSVtRevM10.

5. The method of claim 1, wherein said introducing by particle mediated gene transfer is carried out by introducing particles on which said gene is coated into said plurality of T cells, wherein said particles are made of a material selected from the group consisting of inert metals and inert plastics.

6. The method of claim 5, wherein said particles are made of a material selected from the group consisting of gold, silver, platinum, tungsten, polystyrene, polypropylene, and polycarbonate.

7. The method of claim 5, wherein said particles are gold particles.

8. The method of claim 5, wherein said particles have a diameter of 0.5 to 5 microns.

9. The method of claim 5, wherein said particles have a diameter of 1 to 3 microns.

10. The method of claim 5, wherein said gene is contained in a plasmid and said particles are gold particles, and said plasmid is coated on said particles in an amount of 3 to 30 micrograms of plasmid per milligram of particles.

11. The method of claim 10, wherein said particles are coated with an encapsulating agent before being coated with said plasmid.

12. The method of claim 11, wherein said encapsulating agent is polylysine.

13. The method of claim 1, wherein said gene is introduced into 10^9 to 10^{13} T cells.

14. The method of claim 1, wherein said gene is introduced into 10^{10} to 10^{11} T cells.

15. The method of claim 1, wherein said introducing results in said gene being introduced into 1 to 10% of said plurality of T cells.

16. The method of claim 1, wherein steps (i), (ii), and (iii) are repeated a number of times sufficient to result in the introduction of said gene into 0.1 to 30% of said patient's T cells.

17. The method of claim 1, wherein steps (i), (ii), and (iii) are repeated a number of times sufficient to result in the introduction of such gene into 1 to 15% of said patient's T cells.

18. The method of claim 1, wherein said steps (i), (ii), and (iii) are carried out 1 to 10 times.

19. The method of claim 1, wherein said steps (i), (ii), and (iii) are carried out 2 to 5 times.

20. The method of claim 1, wherein said steps (i), (ii), and (iii) are repeated after 2 to 24 hrs.

L22 ANSWER 6 OF 6 USPATFULL on STN
97:63912 Viral vectors.
Wong-Staal, Flossie, San Diego, CA, United States
Mamounas, Michael, Solana Beach, CA, United States
Poeschla, Eric M., San Diego, CA, United States
Kraus, Gunter, La Jolla, CA, United States
Leavitt, Mark, La Jolla, CA, United States
The Regents of the University of California, Oakland, CA, United States
(U.S. corporation)
US 5650309 19970722
APPLICATION: US 1995-442061 19950516 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A vector comprising biologically active nucleic acid sequences from a first and second virus, wherein said nucleic acid sequences of said first virus comprise cis-active AAV nucleic acids for host cell chromosomal integration, said nucleic acid sequences of said second virus comprise a replication defective, rescuable retroviral genome, and wherein said nucleic acid sequences of said second virus also encodes an anti-viral agent operably linked to an expression control sequence.

2. The vector of claim 1, wherein said nucleic acid sequences from said first virus further comprise nucleic acid sequences for nucleic acid replication and encapsidation of the vector.

3. The vector of claim 1, wherein the second virus is an HIV virus.

4. The vector of claim 1, wherein said cis-active nucleic acid sequences are AAV 5' and 3' ITR regions.

5. The vector of claim 1, wherein the second virus is HIV, and wherein the replication defective, rescuable HIV genome encodes a non-functional gene selected from the **tat**, **rev**, gag, pol, env, v/f, vpr, nef, and vpu/vpx genes.

6. The vector of claim 1, wherein the second virus is HIV, and wherein the replication defective, rescuable HIV genome does not encode a gene selected from the **tat**, **rev**, gag, pol, env, vif, vpr, nef, and vpu/vpx genes.

7. The vector of claim 1, wherein said expression control sequence comprises a constitutive promoter.

8. The vector of claim 1, wherein said expression control sequence comprises an inducible promoter.

9. The vector of claim 1, wherein said expression control sequence comprises an inducible promoter activated in response to viral replication of a replication competent virus corresponding to the replication defective portion of the vector.

10. The vector of claim 1, wherein the anti-viral agent is selected to specifically inhibit the replication of the second virus.

11. The vector of claim 1, wherein said second virus encodes an anti-viral agent selected from the group consisting of an antisense nucleic acid, a ribozyme, a decoy nucleic acid, a **transdominant** gene and a suicide gene.

12. The vector of claim 1, wherein said second virus encodes an anti viral agent selected from the group consisting of an antisense nucleic acid comprising the HIV TAR or RRE sequence, a decoy nucleic acid molecule comprising the TAR sequence or the RRE sequence, a hammerhead ribozyme, and a hairpin ribozyme.

13. The vector of claim 1, further comprising a nucleic acid encoding a selectable marker operatively linked to an expression control sequence.

14. The vector of claim 1, further comprising a second anti-viral agent operatively linked to an expression control sequence.

15. A mammalian cell transduced with a vector comprising biologically active nucleic acid sequences from a first and second virus, wherein said nucleic acid sequences of said first virus comprise cis-active AAV nucleic acids for host cell chromosomal integration, said nucleic acid sequences of said second virus comprise a replication defective, rescuable retroviral genome, and wherein said nucleic acid sequences of said second virus also encodes an anti-viral agent operably linked to an expression control sequence.

16. The mammalian cell of claim 15 wherein the mammalian cell is a

monocyte.

17. A method for inhibiting viral replication in a cell in vitro, comprising transducing the cell with a vector comprising biologically active nucleic acid sequences from a first and second virus, wherein said nucleic acid sequences of said first virus comprise cis-active AAV nucleic acids for host cell chromosomal integration, said nucleic acid sequences of said second virus comprise a replication defective, rescuable retroviral genome, and wherein said nucleic acid sequences of said second virus also encodes an anti-viral agent operably linked to an expression control sequence.

18. The method of claim 17, wherein said transduced cell inhibits viral replication by an HIV virus.

19. The method of claim 17, wherein the cell includes genes necessary for activating an expression control sequence contained within said vector.

20. The method of claim 17, wherein the cell is a hematopoietic stem cell, fetal cord blood cell, T-lymphocyte or monocyte.

21. A method for making anti-viral agents in a cell in vitro, comprising transducing the cell with a vector comprising biologically active nucleic acid sequences from a first and second virus, wherein said nucleic acid sequences of said first virus comprise cis-active nucleic acids encoding viral sequences for host cell chromosomal integration, said nucleic acid sequences of said second virus comprise a replication defective, rescuable viral genome, and wherein said nucleic acid sequences of said second virus encode an anti-viral agent operably linked to an expression control sequence, wherein the cell includes genes necessary for activating said expression control sequence, and culturing the cell under conditions for expression of the anti-viral agent.

=> d his

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     (FILE 'HOME' ENTERED AT 08:23:09 ON 27 JUN 2006)

     FILE 'USPATFULL' ENTERED AT 08:23:19 ON 27 JUN 2006
                E AGUILAR-CORDOVA C E/IN
                E CORDOVA C E A/IN
                E AGUILAR CORDOVA C E/IN
L1              8 S E4-E7
                E BELMONT JOHN W/IN
L2              3 S E3
L3              2 S L2 NOT L1
                E HARPER J WADE/IN
L4              1 S E3
L5              0 S L4 NOT L1

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L6              9 S E2-E5
                E BELMONT J W/IN
L7              2 S E3
L8              1 S L7 NOT L6
                E HARPER J W/IN
L9             10 S E3
L10             9 S L9 NOT L6

     FILE 'USPATFULL' ENTERED AT 08:27:55 ON 27 JUN 2006
                E AGUILAR CORDOVA C E/AU

     FILE 'MEDLINE' ENTERED AT 08:28:23 ON 27 JUN 2006
                E AGUILAR CORDOVA C E/AU
L11            77 S E2-E6
L12             5 S L11 AND (TAT OR REV)
                E BELMONT J W/AU
L13           104 S E3 OR E4 OR E6
L14            99 S L13 NOT L11
L15             0 S L14 AND (TAT OR REV)
                E HARPER J W/AU
L16           148 S E3-E5
L17           147 S L16 NOT L11
L18             1 S L17 AND (TAT OR REV)

     FILE 'USPATFULL' ENTERED AT 08:32:37 ON 27 JUN 2006
L19         13995 S (TAT AND REV)
L20           296 S L19 AND TRANSDOMINANT
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L22 6 S L21 AND AY<1996

=> file medline
COST IN U.S. DOLLARS SINCE FILE ENTRY TOTAL SESSION
FULL ESTIMATED COST 25.15 63.27

FILE 'MEDLINE' ENTERED AT 08:35:23 ON 27 JUN 2006

FILE LAST UPDATED: 24 JUN 2006 (20060624/UP). FILE COVERS 1950 TO DATE.

On December 11, 2005, the 2006 MeSH terms were loaded.

The MEDLINE reload for 2006 is now (26 Feb.) available. For details on the 2006 reload, enter HELP RLOAD at an arrow prompt (=>).
See also:

http://www.nlm.nih.gov/mesh/
http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

OLDMEDLINE is covered back to 1950.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2006 vocabulary.

This file contains CAS Registry Numbers for easy and accurate substance identification.

=> s (Tat and Rev)
7253 TAT
5692 REV
L23 657 (TAT AND REV)

=> s l23 and (transdominant or trans-dominant)
347 TRANSDOMINANT
96000 TRANS
93294 DOMINANT
344 TRANS-DOMINANT
(TRANS(W)DOMINANT)
L24 24 L23 AND (TRANSDOMINANT OR TRANS-DOMINANT)

=> d l24,cbib,ab,1-24

L24 ANSWER 1 OF 24 MEDLINE on STN
2006055526. PubMed ID: 16439542. Novel Pol II fusion promoter directs human immunodeficiency virus type 1-inducible coexpression of a short hairpin RNA and protein. Unwalla Hoshang J; Li Hai-Tang; Bahner Ingrid; Li Ming-Jie; Kohn Donald; Rossi John J. (Department of Molecular Biology, Beckman Research Institute of the City of Hope, 1450 E. Duarte Rd., Duarte, CA 91010, USA.. jrossi@coh.org) . Journal of virology, (2006 Feb) Vol. 80, No. 4, pp. 1863-73. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB We demonstrate a novel approach for coexpression of a short hairpin RNA (shRNA) with an open reading frame which exploits transcriptional read-through of a minimal polyadenylation signal from a Pol II promoter. We first observed efficient inducible expression of enhanced green fluorescent protein along with an anti-**rev** shRNA. We took advantage of this observation to test coexpression of the **transdominant** negative mutant (humanized) of human immunodeficiency type 1 (HIV-1) **Rev** (huRevM10) along with an anti-**rev** shRNA via an HIV-1-inducible fusion promoter. The coexpression of the shRNA and **transdominant** protein resulted in potent, long-term inhibition of HIV-1 gene expression and suppression of shRNA-resistant mutants. This dual expression system has broad-based potential for other shRNA applications, such as cases where simultaneous knockdown of mutant and wild-type transcripts must be accompanied by replacement of the wild-type protein.

L24 ANSWER 2 OF 24 MEDLINE on STN
2003607758. PubMed ID: 14689754. Gene therapy for human immunodeficiency virus infection in the humanized SCID mouse model. Touraine Jean-Louis; Sanhadji Kamel; Sembeil Rachel. (Immunology Laboratory for Transplantation and Immunovirology, Edouard Herriot Hospital, Lyon, France.. jean-louis.touraine@chu-lyon.fr) . The Israel Medical Association journal : IMAJ, (2003 Dec) Vol. 5, No. 12, pp. 863-7. Journal code: 100930740. ISSN: 1565-1088. Pub. country: Israel. Language: English.
AB BACKGROUND: The humanized SCID mouse model is an attractive tool for testing gene therapy to combat human immunodeficiency virus infection in vivo. OBJECTIVES: To devise a more specific gene therapy directed against HIV, replacing the formerly used interferon with either soluble CD4 molecule immunoadhesin (sCD4-IgG) and/or anti-gp41 monoclonal antibody

Human monocytoid cell line (U937) was transfected with IFN alpha, beta or gamma genes. 3T3 murine fibroblastic cell line was transfected with sCD4-IgG or 2F5, or both genes, and a human T4 cell line (CEM) was grafted to SCID mice. Negative **transdominant** genes (**Tat**, **Rev** or both) were also transduced in CEM T cell line. Animals were then challenged with HIV-1, Viral load was followed. RESULTS: IFN alpha or beta were potent anti-HIV, reducing viral load in vivo and inhibiting reverse transcriptase activity in human-removed cells from animals. sCD4-IgG immunoadhesin and gp41 monoclonal antibody resulted in a dramatic reduction of HIV-1 cellular and plasmatic viral load in humanized SCID mice. The simultaneous introduction of negative **Tat** and **Rev** genes resulted in a synergistic inhibition of HIV-1 replication in vivo. CONCLUSIONS: Despite the marked reduction of HIV-1 propagation by IFN genes or by negative **Tat** and **Rev** transdominants, the gene therapy using soluble CD4 immunoadhesin or anti-gp41 was a more efficient preventive treatment against HIV infection.

L24 ANSWER 3 OF 24 MEDLINE on STN

2002211112. PubMed ID: 11938457. Inhibition of HIV-1 replication by novel lentiviral vectors expressing **transdominant Rev** and HIV-1 env antisense. Mautino M R; Morgan R A. (Clinical Gene Therapy Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA.) Gene therapy, (2002 Apr) Vol. 9, No. 7, pp. 421-31. Journal code: 9421525. ISSN: 0969-7128. Pub. country: England: United Kingdom. Language: English.

AB Retroviral vectors expressing **transdominant** negative mutants of **Rev** (TdRev) inhibit HIV-1 replication by preventing the nuclear export of unspliced viral transcripts, thus inhibiting the synthesis of Gag-Pol, Env and reducing the levels of genomic RNA available for packaging. Due to these effective mechanisms of inhibition, production of HIV-1-based lentiviral vectors expressing TdRev has been difficult. Here we describe HIV-based vectors in which expression of TdRev is negatively regulated by **Rev** expression. In these vectors, we maintained the wild-type HIV-1 **Tat**/**Rev** exons and intron configuration and its mode of splicing regulation. The second **Rev** exon was mutated to encode TdRev. Inhibition of TdRev expression by **Rev** during vector production yields high titer vector preparations. A second vector containing an additional anti-HIV gene (env-antisense) was constructed by flipping a 1.2-kb env fragment contained within the **Tat**/TdRev intron. SupT1 cells and primary CD4+ lymphocytes transduced with these vectors inhibit HIV-1 replication and show a preferential advantage for survival. Although these vectors are poorly mobilized to secondary target cells by wild-type HIV-1, they reduce the infectivity of the wild-type virions escaping inhibition.

L24 ANSWER 4 OF 24 MEDLINE on STN

2002087344. PubMed ID: 11815282. A combination anti-HIV-1 gene therapy approach using a single transcription unit that expresses antisense, decoy, and sense RNAs, and **trans-dominant** negative mutant Gag and Env proteins. Ding Shi-Fa; Lombardi Rocco; Nazari Reza; Joshi Sadhna. (Department of Medical Genetics and Microbiology, Faculty of Medicine, University of Toronto, Toronto, Ontario M5S 3E2, Canada.) Frontiers in bioscience : a journal and virtual library, (2002 Feb 1) Vol. 7, pp. a15-28. Electronic Publication: 2002-02-01. Journal code: 9709506. E-ISSN: 1093-4715. Pub. country: United States. Language: English.

AB Oncoretroviral vectors were engineered to allow constitutive expression of an antisense RNA and the trans-activator of transcription (**Tat**)-inducible expression of a mRNA containing the trans-activation response (TAR) element, the **Rev** response element (RRE), and the efficient packaging signal (Psi(e) of human immunodeficiency virus-1 (HIV-1) RNA. Nuclear export of this mRNA by the regulator of expression of virion proteins (**Rev**) would allow its translation into wild type (WT) (MoTN-Ti-GE-Ri- Ter) or **trans-dominant** negative mutant (TDM) (MoTN-Ti-GmEm-Ri-Ter) Gag and Env proteins. Thus, the antisense RNA produced in a constitutive manner would ensure that even if there is leaky expression, no WT/TDM Gag or Env protein would be produced in the uninfected cells. If cells become infected by HIV-1, the antisense RNA would inhibit HIV-1 replication. Failure on the part of antisense RNA to inhibit virus replication would allow GE/GmEm mRNA production. The GE/GmEm mRNA would cause partial inhibition of HIV-1 replication as it contains the TAR, RRE, and Psi(e) signal sequences. Translation of GmEm mRNA would give rise to TDM Gag and Env proteins, which would further decrease progeny virus infectivity. **Tat**- and **Rev**-inducibility was demonstrated in transfected HeLa and HeLa-Tev cells. Full-length WT/TDM Gag production was confirmed by Western blot analysis. Amphotropic vector particles were used to transduce a human CD4+ T-lymphoid cell line, and the stable transductants were challenged with HIV-1. Virus replication was better inhibited by the MoTN-Ti-GE-Ri-Ter vector than by the MoTN-Ti-GmEm-Ri-Ter vector. Inhibition of HIV-1 replication was also demonstrated in transduced CD4+ human peripheral blood T lymphocytes (PBLs). Moreover, our results suggest that cloning in the reverse transcriptional orientation must be avoided to prevent antisense RNA-mediated inhibition of transgene and endogenous gene expression.

L24 ANSWER 5 OF 24 MEDLINE on STN
2001166395. PubMed ID: 11264348. Inhibition of human immunodeficiency virus type 1 (HIV-1) replication by HIV-1-based lentivirus vectors expressing **transdominant Rev**. Mautino M R; Keiser N; Morgan R A. (Clinical Gene Therapy Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland 20892-1851, USA.) Journal of virology, (2001 Apr) Vol. 75, No. 8, pp. 3590-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Retrovirus vectors expressing **transdominant**-negative mutants of **Rev** (TdRev) inhibit human immunodeficiency virus type 1 (HIV-1) replication by preventing the nuclear export of unspliced viral transcripts, thus inhibiting the synthesis of Gag-Pol, Env, and genomic RNA. The use of HIV-1-based vectors to express TdRev would have the advantage of allowing access to nondividing hematopoietic cells. It would also provide additional levels of protection by sequestering the viral regulatory proteins **Tat** and **Rev**, competing for encapsidation into wild-type virions, and inhibiting reverse transcription. Here we describe HIV-1-based vectors that express TdRev. These vectors contain mutations in the splicing signals or replacement of the **Rev**-responsive element by the simian retrovirus type 1 constitutive transport element, making them less sensitive to the inhibitory effects of TdRev. In addition, overexpression of **Rev** and the use of an HIV-1 helper plasmid that drives high levels of Gag-Pol synthesis were used to transiently overcome the inhibition by TdRev of the synthesis of Gag-Pol during vector production. SupT1 cells transduced with these vectors were more resistant to HIV-1 replication than cells transduced with Moloney murine leukemia virus-based vectors expressing TdRev. Furthermore, we show that these vectors can be mobilized by the wild-type virus, reducing the infectivity of virions escaping inhibition and conferring protection against HIV-1 replication to previously untransduced cells.

L24 ANSWER 6 OF 24 MEDLINE on STN
1999383787. PubMed ID: 10456787. Inhibition of HIV/SIV replication by dominant negative Gag mutants. Shimano R; Inubushi R; Oshima Y; Adachi A. (Department of Virology, The University of Tokushima School of Medicine, Japan.) Virus genes, (1999) Vol. 18, No. 3, pp. 197-201. Journal code: 8803967. ISSN: 0920-8569. Pub. country: United States. Language: English.

AB There are several major strategies against HIV/AIDS. Of these, the gene therapy is a novel, challenging, and promising one. The target genes, which have been extensively studied for the potential gene therapy of HIV/AIDS, include those of cellular and viral origins. Especially, **trans-dominant** negative **Tat**, **Rev**, Env, Pol, and Gag mutants of HIV have currently attracted considerable attention. In this brief review, we summarize the nature of the HIV/SIV mutants of this category and discuss their future use for gene therapy with special reference to the dominant negative Gag mutants of HIV-1.

L24 ANSWER 7 OF 24 MEDLINE on STN
1999145133. PubMed ID: 10022535. Inhibition of human immunodeficiency virus type 1 by **Tat/Rev**-regulated expression of cytosine deaminase, interferon alpha2, or diphtheria toxin compared with inhibition by **transdominant Rev**. Ragheb J A; Couture L; Mullen C; Ridgway A; Morgan R A. (National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA.) Human gene therapy, (1999 Jan 1) Vol. 10, No. 1, pp. 103-12. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB A retroviral vector was designed to express toxic proteins only in the presence of the HIV-1 **Rev** and/or **Tat** protein(s). The design of this vector incorporates an HIV-specific expression cassette that consists of three elements: the U3R region of the HIV-1 IIIB LTR provides the promoter and **Tat**-responsive element, a modified intron derived from the human c-src gene facilitates the splicing of inserted genes, and the HIV-1 RRE region enhances the transport of unspliced mRNAs. To further limit potential readthrough transcription, the expression cassette was inserted in the reverse transcriptional orientation relative to the retroviral vector LTR. Three different genes, interferon alpha2, diphtheria toxin (DT-A), and cytosine deaminase, were inserted into this vector. **Tat** and **Rev** inducibility was demonstrated directly by a >300-fold induction of interferon production and functionally by a decrease in colony-forming units when a **Tat** and **Rev** expression vector was titered on HeLa cells harboring the inducible DT-A cassette. The **Tat**-inducible cytosine deaminase gene was tested in the Sup-T1 T cell line and shown to inhibit HIV-1 production only when engineered cells were grown in the presence of 5-fluorocytosine. To test the ability of this system to inhibit HIV-1 infection in bulk PBL cultures, a series of transduction and challenge experiments was initiated with both the interferon and DT-A vectors. Protection against infection was documented against three HIV strains in PBLs. Last, the interferon and DT-A vectors were compared with a vector encoding a **transdominant Rev** protein and were shown to mediate equal or greater inhibition of HIV-1.

L24 ANSWER 8 OF 24 MEDLINE on STN

HIV-1 **Rev** through an alternative RxRE-independent pathway mediated by the RU5 portion of the 5'-LTR. Kubota S; Furuta R A; Hatanaka M; Pomerantz R J. (Dorrance H. Hamilton Laboratories, Department of Medicine, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107, USA.) Biochemical and biophysical research communications, (1998 Feb 4) Vol. 243, No. 1, pp. 79-85. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB The 5'-RU5 portion of human T-lymphocyte virus type I (HTLV-I) long terminal repeat (LTR) had been reported to contain cis-acting elements for the controlled viral gene expression by the rex gene product. In this study, the human immunodeficiency virus type I (HIV-1) **Rev** protein was found to enhance gene expression, acting through the 5'-RU5 portion of HTLV-I, while the Rex-responsive element (RxRE)-mediated activation by **Rev** was reconfirmed to be negative. This positive action of HIV-1 **Rev** on HTLV-I gene expression seemed to be distinct from the widely accepted Rex or **Rev** function to facilitate the nuclear export of RxRE-containing unspliced viral mRNAs, since a **trans-dominant**, nuclear export-deficient mutant (RevM10) still retained the RU5-mediated effector function. Analyses of the functional aspects of **Tat/Rev** fusion proteins on the HTLV-I RU5 suggested a specific interaction of **Rev** and RU5, but lacked evidence for the binding of **Rev** to the RU5 at the RNA level. These results suggest an answer to the controversy regarding a Rex-like function occasionally observed with HIV-1 **Rev** and its related proteins. It may also be suggested that particular care should be taken when such a **trans-dominant Rev** mutant is considered to be used as a genetic therapy against HIV-I infection, in individuals infected with both HIV-I and HTLV-1.

L24 ANSWER 9 OF 24 MEDLINE on STN

1998139081. PubMed ID: 9499041. Comparative analyses of intracellularly expressed antisense RNAs as inhibitors of human immunodeficiency virus type 1 replication. Veres G; Junker U; Baker J; Barske C; Kalfoglou C; Ilves H; Escaich S; Kaneshima H; Bohnlein E. (Systemix Inc., a Novartis Company, Palo Alto, California 94304, USA.. gveres@stem.com) . Journal of virology, (1998 Mar) Vol. 72, No. 3, pp. 1894-901. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The antiviral activities of intracellularly expressed antisense RNAs complementary to the human immunodeficiency virus type 1 (HIV-1) pol, vif, and env genes and the 3' long terminal repeat (LTR) sequence were evaluated in this comparative study. Retroviral vectors expressing the antisense RNAs as part of the Moloney murine leukemia virus LTR promoter-directed retroviral transcript were constructed. The CD4+ T-cell line CEM-SS was transduced with retroviral constructs, and Northern blot analyses showed high steady-state antisense RNA expression levels. The most efficient inhibition of HIV-1 replication was observed with the env antisense RNA, followed by the pol complementary sequence, leading to 2- to 3-log10 reductions in p24 antigen production even at high inoculation doses (4 x 10(4) 50% tissue culture infective doses) of the HIV-1 strain HXB3. The strong antiviral effect correlated with a reduction of HIV-1 steady-state RNA levels, and with intracellular **Tat** protein production, suggesting that antisense transcripts act at an early step of HIV-1 replication. A lower steady-state antisense RNA level was detected in transduced primary CD4+ lymphocytes than in CEM-SS cells. Nevertheless, replication of the HIV-1 JR-CSF isolate was reduced with both the pol and env antisense RNA. Intracellularly expressed antisense sequences demonstrated more pronounced antiviral efficacy than the **transdominant** RevM10 protein, making these antisense RNAs a promising gene therapy strategy for HIV-1.

L24 ANSWER 10 OF 24 MEDLINE on STN

97319610. PubMed ID: 9176513. Studies on the effect of the combined expression of anti-**tat** and anti-**rev** genes on HIV-1 replication. Caputo A; Rossi C; Bozzini R; Betti M; Grossi M P; Barbanti-Brodano G; Balboni P G. (Institute of Microbiology, School of Medicine, University of Ferrara, Italy.) Gene therapy, (1997 Apr) Vol. 4, No. 4, pp. 288-95. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A series of retroviral vectors with potential anti-**tat** and antirev activity was developed. Vectors containing a **tat transdominant** negative mutant (tat22/37) and an RRE decoy in different positions, directed by the same promoter or by different promoters, were generated. Retroviral vectors containing tat22/37 and the RevM10 **transdominant** negative mutant were also constructed. Jurkat cells were transduced with the recombinant retroviruses to produce monoclonal and polyclonal cultures. In these cell lines the recombinant proviruses were correctly integrated and expression of the inserted genes was detected by Northern blot or RT-PCR analysis. However, infection of these cell lines with HIV-1 showed that none of these recombinant constructs inhibited virus replication at a high multiplicity of infection (MOI). At a low MOI, two cell clones containing tat22/37 and the RRE decoy in 3' position showed a long lasting protection against virus replication, in comparison to control cultures expressing tat22/37 or RRE alone. Combination of **tat**

low and high MOIs. At a low MOI, HIV-1 replication was efficiently blocked in two cell clones expressing the RevM10 mutant alone. These results show a synergic effect of anti-**tat** and anti-**rev** molecules when the RRE sequence is cloned 3' to tat22/37, suggesting the possibility of using this vector design to control HIV-1 replication.

L24 ANSWER 11 OF 24 MEDLINE on STN

97288989. PubMed ID: 9143912. Protection of primary human T cells from HIV infection by Trev: a **transdominant** fusion gene. Chinen J; Aguilar-Cordova E; Ng-Tang D; Lewis D E; Belmont J W. (Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030, USA.) Human gene therapy, (1997 May 1) Vol. 8, No. 7, pp. 861-8. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB Gene therapy is one of several approaches that are being tested in the search for an effective anti-human immunodeficiency virus (HIV) treatment. In this strategy, a "protective" gene would be introduced into target cells, rendering them relatively resistance to the virus-induced cytopathicity. **Tat** and **Rev** are viral proteins essential for HIV gene expression. **Tat** increases viral gene transcription and **Rev** is responsible for the nuclear export of mRNA encoding structural viral proteins. A fusion protein (Trev) was constructed, joining **Tat** and **Rev transdominant** mutant gene sequences. Previously, we showed that Trev inhibits both **Tat** and **Rev** activities in Jurkat T cells. To determine whether Trev could inhibit HIV replication in primary cells, we transferred the trev gene to peripheral blood lymphocytes and challenged them with different HIV strains. Levels of HIV p24 antigen (Ag) were reduced 4- to 15-fold in cultures of Trev-CD4+ T cells infected with two HIV primary clinical isolates and were not detectable in cultures infected with HIV strains NL4-3 and SF2. In contrast, cultures of nontransduced CD4+ T cells infected with the same viruses had levels of HIV p24 Ag up to 10 ng/ml. Trev-transduced CD4+ T cells demonstrated increased survival following HIV challenge for the length of the experiments (30 days). We did not observe rapid emergence of Trev-resistant HIV in our cultures. Following HIV challenge, cell-associated Trev protein was increased, supporting the hypothesis that cells surviving Trev expression provided a cell survival advantage. This work showed that Trev was able to inhibit HIV replication in primary CD4+ T cells, and, therefore the trev gene could be a candidate for gene therapy against HIV.

L24 ANSWER 12 OF 24 MEDLINE on STN

97234667. PubMed ID: 9116267. Inhibition of human immunodeficiency virus-1 (HIV-1) replication after transduction of granulocyte colony-stimulating factor-mobilized CD34+ cells from HIV-1-infected donors using retroviral vectors containing anti-HIV-1 genes. Bauer G; Valdez P; Kearns K; Bahner I; Wen S F; Zaia J A; Kohn D B. (Department of Pediatrics, University of Southern California School of Medicine, Los Angeles, USA.) Blood, (1997 Apr 1) Vol. 89, No. 7, pp. 2259-67. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Transfer of "anti-HIV-1 genes" into hematopoietic stem cells of human immunodeficiency virus-1 (HIV-1)-infected individuals may be a potent therapeutic approach to render mature cells arising from transduced stem cells resistant to the destructive events associated with HIV-1 infection. To determine the feasibility of gene therapy for acquired immunodeficiency syndrome in individuals already infected with HIV-1, granulocyte colony-stimulating factor mobilized peripheral blood CD34+ cells were isolated from HIV-1-infected individuals and transduced with retroviral vectors containing three different anti-HIV-1-genes: the **Rev** binding domain of the **Rev** Responsive Element (RRE decoy) (L-RRE-neo), a double hammerhead ribozyme vector targeted to cleave the **tat** and **rev** transcripts (L-TR/**TAT**-neo), and the **trans-dominant** mutant of **rev** (M10) (L-M10-SN). As a control, a vector mediating only neomycin resistance (LN) was used. After 3 days of transduction on allogeneic stroma in the presence of stem cell factor, interleukin-6 (IL-6), and IL-3, the cultures were G418-selected, and then challenged with HIV-1(JR-FL) and a primary HIV-1 isolate. Compared with the control cultures, the L-RRE-neo-, L-TR/**TAT**-neo-, and L-M10-SN-transduced cultures displayed up to 1,000-fold inhibition of HIV-1 replication after challenge with HIV-1(JR-FL) and the primary HIV-1 isolate. Growth of the hematopoietic cells in long-term bone marrow culture was not perturbed by the presence of any of the anti-HIV-1 genes. This study shows that anti-HIV-1 genes can be introduced into CD34+ cells from individuals already infected with HIV-1, and strongly inhibit HIV-1 replication in primary monocytes derived from the CD34+ progenitors.

L24 ANSWER 13 OF 24 MEDLINE on STN

96256811. PubMed ID: 8676525. Inhibition of human immunodeficiency virus type 1 replication is enhanced by a combination of **transdominant Tat** and **Rev** proteins. Ulich C; Harrich D; Estes P; Gaynor R B. (Division of Molecular Virology, Department of Medicine, University of Texas Southwestern Medical Center at Dallas, Texas 75235-8594, USA.) Journal of virology, (1996 Jul) Vol. 70, No. 7, pp. 4871-6. Journal code: 0113724.

AB Mutation of either of two critical human immunodeficiency virus type 1 (HIV-1) regulatory proteins, **Tat** and **Rev**, results in marked defects in viral replication. Thus, inhibition of the function of one or both of these proteins can significantly inhibit viral growth. In the present study, we constructed a novel **transdominant Tat** mutant protein and compared its efficiency in inhibiting HIV-1 replication with that of **transdominant** mutant **Rev** M10 when these proteins were stably expressed either alone or in combination in T-lymphocyte cell lines. The **transdominant Tat** mutant protein alone resulted in a modest inhibition of HIV replication, but it was able to enhance the ability of the M10 **Rev** mutant protein to inhibit HIV-1 replication. These results suggest a possible synergistic effect of these **transdominant** mutant proteins in inhibiting HIV-1 replication.

L24 ANSWER 14 OF 24 MEDLINE on STN
96238293. PubMed ID: 8787346. [Gene therapy for hereditary and acquired human diseases]. Therapie genique de maladies humaines hereditaires et acquises. Mehtali M; Imler J L; Sorg T; Pavirani A. (TRANSGENE S.A., Strasbourg.) Annales d'endocrinologie, (1995) Vol. 56, No. 6, pp. 571-4. Ref: 15. Journal code: 0116744. ISSN: 0003-4266. Pub. country: France. Language: French.

AB Cystic Fibrosis (CF) and AIDS are primary candidate disorders to be treated by gene therapy, owing to their lethality and the absence of efficient clinical treatments. Treatment of CF by gene therapy will require the transfer of the functional CFTR cDNA into the diseased human airway epithelia since mutations within the CFTR gene are responsible for CF. We have therefore cloned the human CFTR cDNA and developed a recombinant E1-deleted adenoviral vector carrying a CFTR expression cassette. We demonstrated in vitro the ability of this vector to efficiently transduce human lung cells isolated from CF patients and to correct their phenotype. Efficient in, vivo delivery of the CFTR cDNA to the airways of cotton rats and rhesus monkeys was also obtained and no dissemination of the recombinant viral vector in other tissues than the airways was observed. We have therefore designed a phase I clinical trial involving CF patients. In contrast to the monogenic CF disease, the mechanisms of AIDS pathogenesis still remain poorly understood. Such limited knowledge of the disease constitutes a serious restriction to the development of a rational gene therapy strategy for AIDS. Since HIV, the causative agent of AIDS, predominantly infects cells of the hematopoietic system, pluri- or multipotent stem cells may constitute potential targets for the introduction of a foreign anti-HIV gene that will inhibit HIV replication and/or spread. Reimplantation of the genetically modified stem cells into asymptomatic HIV-infected patients should theoretically allow the repopulation of the host's immune system with mature CD4+ cells expressing novel molecules that interfere with viral replication, thus slowing the progression of AIDS. We identified several new **transdominant** inhibitors derived from the viral **TAT** and **REV** proteins and showed their ability to confer to human CD4 lymphocytes resistance against HIV1 infection. Retroviral vectors carrying these potential therapeutic genes have been developed and are currently being tested in vivo in newly developed transgenic animal models, in humanized SCID mice and in macaques.

L24 ANSWER 15 OF 24 MEDLINE on STN
96050917. PubMed ID: 7584057. Regulated expression of a dominant negative form of **Rev** improves resistance to HIV replication in T cells. Liu J; Woffendin C; Yang Z Y; Nabel G J. (Howard Hughes Medical Institute, University of Michigan Medical Center, Department of Internal Medicine, Ann Arbor 48109-0650, USA.) Gene therapy, (1994 Jan) Vol. 1, No. 1, pp. 32-7. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Infection by the human immunodeficiency virus (HIV) has remained refractory to treatment, and molecular genetic interventions have been developed for the treatment of the acquired immunodeficiency syndrome (AIDS). Previous studies have focused on the development of gene products which inhibit productive HIV replication, including **transdominant** proteins, RNA decoys and ribozymes. In this report, we show that appropriate expression vectors which optimize production and regulated synthesis of a **transdominant** mutant form of **Rev** improve its antiviral effect. The combination of a strong constitutive enhancer, a **Tat** activation response (TAR) regulatory element and **transdominant Rev** take advantage of three aspects of early viral gene expression to confer increased resistance to HIV replication. This vector may be useful, alone or in combination with other antiviral genes, to provide gene therapy for AIDS.

L24 ANSWER 16 OF 24 MEDLINE on STN
95339003. PubMed ID: 7614248. Inhibition of HIV-1 by a double **transdominant** fusion gene. Aguilar-Cordova E; Chinen J; Donehower L A; Harper J W; Rice A P; Butel J S; Belmont J W. (Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030, USA.) Gene therapy, (1995 May) Vol. 2, No. 3, pp. 181-6. Journal code: 9421525. ISSN: 0969-7128.

AB A double **transdominant** fusion gene (trev) designed to inhibit two essential HIV functions simultaneously was constructed by linking **tat** and **rev transdominant** mutants. Trev independently inhibited both **Tat** and **Rev** functions, localized within the nucleus and cells transfected with trev showed a stable inhibition of HIV-1-mediated cytopathicity. A retroviral vector of trev was made and shown also to confer protection from HIV cytopathic effects. Simultaneous inhibition of two essential viral genes presents significant advantages for potential gene therapy treatment of HIV infection over conventional single effect molecules.

L24 ANSWER 17 OF 24 MEDLINE on STN
95287453. PubMed ID: 7769662. Inhibition of clinical human immunodeficiency virus (HIV) type 1 isolates in primary CD4+ T lymphocytes by retroviral vectors expressing anti-HIV genes. Vandendriessche T; Chuah M K; Chiang L; Chang H K; Ensoli B; Morgan R A. (Clinical Gene Therapy Branch, National Cancer Institute, Bethesda, Maryland 20892, USA.) Journal of virology, (1995 Jul) Vol. 69, No. 7, pp. 4045-52. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Gene therapy may be of benefit in human immunodeficiency virus type 1 (HIV-1)-infected individuals by virtue of its ability to inhibit virus replication and prevent viral gene expression. It is not known whether anti-HIV-1 gene therapy strategies based on antisense or **transdominant** HIV-1 mutant proteins can inhibit the replication and expression of clinical HIV-1 isolates in primary CD4+ T lymphocytes. We therefore transduced CD4+ T lymphocytes from uninfected individuals with retroviral vectors expressing either HIV-1-specific antisense-TAR or antisense-**Tat/Rev** RNA, **transdominant** HIV-1 **Rev** protein, and a combination of antisense-TAR and **transdominant Rev**. The engineered CD4+ T lymphocytes were then infected with four different clinical HIV-1 isolates. We found that replication of all HIV-1 isolates was inhibited by all the anti-HIV vectors tested. Greater inhibition of HIV-1 was observed with **transdominant Rev** than with antisense RNA. We hereby demonstrated effective protection by antisense RNA or **transdominant** mutant proteins against HIV-1 infection in primary CD4+ T lymphocytes using clinical HIV-1 isolates, and this represents an essential step toward clinical anti-HIV-1 gene therapy.

L24 ANSWER 18 OF 24 MEDLINE on STN
94107961. PubMed ID: 8280800. The development and testing of retroviral vectors expressing **trans-dominant** mutants of HIV-1 proteins to confer anti-HIV-1 resistance. Liem S E; Ramezani A; Li X; Joshi S. (University of Toronto, Department of Microbiology, Ontario, Canada.) Human gene therapy, (1993 Oct) Vol. 4, No. 5, pp. 625-34. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB **Trans-dominant** mutants of human immunodeficiency virus type 1 (HIV-1) **Tat** and **Rev** are attractive candidates for use in gene therapy in the treatment of HIV-1 infections because both are essential for viral replication. Retroviral vectors were constructed to allow either **Tat**-inducible or **Tat**- and **Rev**-inducible expression of **trans-dominant** mutants of **Tat** and **Rev**. These vectors were used to infect a human CD4+ lymphocyte-derived cell line, MT4. To determine the efficacy of various **Tat** and **Rev** mutants in inhibiting HIV-1 multiplication, MT4 cells containing mutant-expressing constructs were infected with HIV-1, and the amount of HIV-1 released in the culture medium was measured for up to 30 days. A high level of resistance was observed in cells expressing the double **tat/rev** mutant in a **Tat**-inducible manner.

L24 ANSWER 19 OF 24 MEDLINE on STN
94079809. PubMed ID: 8257633. Human immunodeficiency virus type 2 (HIV-2) trans-activator (**Tat**): functional domains and the search for **trans-dominant** negative mutants. Arya S K. (Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.) AIDS research and human retroviruses, (1993 Sep) Vol. 9, No. 9, pp. 839-48. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB Human immunodeficiency virus type 2 (HIV-2) trans-activator (**Tat**) is an important trans-regulator of viral gene expression. It differs from the related HIV-1 **Tat** in certain aspects of its structure and function. HIV-2 **Tat** is composed of 130 amino acids versus 86 amino acids for HIV-1 **Tat**. Apart from certain conserved regions, there is little homology between the two Tats. They also differ in their ability to trans-activate HIV-2 and HIV-1 long terminal repeat (LTR)-directed gene expression. As an aid to understanding its mechanism of action, the functional domains important for HIV-2 **Tat** trans-activation of HIV-2 and HIV-1 LTR-directed gene expression were investigated. Like HIV-1 **Tat**, HIV-2 **Tat** contains conserved cysteine- and arginine-rich domains important for its function. However, HIV-2 **Tat** differs from HIV-1 **Tat** in that about 20% of the HIV-2 **Tat** at the amino terminus was not essential for its trans-activation function while HIV-1 **Tat** amino terminus is reportedly a

carboxy terminus of HIV-2 **Tat** was not essential. A domain critical for HIV-2 **Tat**-mediated trans-activation was located just upstream of the cysteine-rich domain. This segment is predicted to adopt an alpha-helical conformation and also contains acidic amino acid residues; thus, it may resemble amphipathic helix-type activation domains found in some transcriptional factors. A region with predicted hydrophobic alpha-helical character located between the cysteine- and arginine-rich domains was also important for HIV-2 **Tat** function. HIV-2 **Tat** mutants that were analogs of HIV-1 **Tat** **trans-dominant** negative mutants did not display such a phenotype.

L24 ANSWER 20 OF 24 MEDLINE on STN
93267767. PubMed ID: 8388497. Comparison of **trans-dominant** inhibitory mutant human immunodeficiency virus type 1 genes expressed by retroviral vectors in human T lymphocytes. Bahner I; Zhou C; Yu X J; Hao Q L; Guatelli J C; Kohn D B. (Division of Research Immunology/Bone Marrow Transplantation, Childrens Hospital Los Angeles, California.) Journal of virology, (1993 Jun) Vol. 67, No. 6, pp. 3199-207. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB **trans-Dominant** inhibitory mutant versions of the human immunodeficiency virus type 1 (HIV-1) regulatory genes **tat** and **rev** have previously been described. We have constructed a series of retroviral vectors to transduce these genes and compare their inhibitory activities. The inhibitory activities were measured with transient transfection assays by using a reporter which expresses an HIV-1 gag-Escherichia coli lacZ fusion protein with strict dependence on coexpression of both **tat** and **rev**. Additionally, the vectors were packaged as amphotropic virions and used to stably transduce human CEM T lymphocytes. The transduced CEM cells were challenged with HIV-1, and the effects of the mutant HIV-1 genes were determined by measuring the levels of HIV-1 p24gag produced. A **tat** gene substituted at amino acid 41 (tatk41a) retained partial trans-activating activity and lacked inhibitory activity. A **tat** gene with a premature stop codon at amino acid 54 (tat54ter) showed moderate **trans-dominant** inhibition of the reporter plasmid but failed to significantly inhibit HIV-1 replication. The M10 **rev** mutant, with a 2-amino-acid substitution, showed strong **trans-dominant** inhibitory activity both in the reporter plasmid and in the HIV-1 infection assay. The greatest inhibition of HIV-1 growth was seen when M10 was expressed under the transcriptional control of a human cytomegalovirus promoter; slightly less inhibition was achieved when expression of M10 was controlled by the Moloney murine leukemia virus long terminal repeat, and minimal inhibition was seen when the HIV-1 long terminal repeat controlled the M10 gene. These results demonstrate the potential utility of retroviral vectors expressing **trans-dominant** inhibitory mutant HIV-1 genes for gene therapy approaches to AIDS.

L24 ANSWER 21 OF 24 MEDLINE on STN
93028565. PubMed ID: 1409715. Inhibition of human immunodeficiency virus type 1 replication in human T cells by retroviral-mediated gene transfer of a dominant-negative **Rev** trans-activator. Bevec D; Dobrovnik M; Hauber J; Bohnlein E. (Sandoz Research Institute, Vienna, Austria.) Proceedings of the National Academy of Sciences of the United States of America, (1992 Oct 15) Vol. 89, No. 20, pp. 9870-4. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Human immunodeficiency virus type 1 (HIV-1) is the causative agent of the acquired immunodeficiency syndrome (AIDS). Currently, no satisfactory treatment for this viral disease is available. Somatic gene therapy has been proposed as an alternative to conventional therapies. Several antiviral gene therapy approaches including ribozymes, antisense inhibition, and RNA-decoy strategies, as well as dominant-negative mutants of HIV-1 proteins (Gag, **Tat**, and **Rev**) have been suggested. To prove the concept of **trans-dominant** inhibition of HIV-1 replication, we transduced CEM cells with a retroviral vector encoding a dominant-negative **rev** gene. Amplification of integrase-specific proviral sequences from high molecular weight DNA indicated successful HIV-1 human T-lymphotropic virus type IIIB (HTLV-IIIB) infection of all cells. In contrast to CEM cells and CEM cells expressing the **rev** wild-type (wt) gene, infection of two CEM-RevM10 clones with HIV-1 did not result in the release of significant levels of p24 Gag antigen as measured by antigen capture assay, indicating a block in HIV-1 replication due to the presence of the **trans-dominant** **Rev** protein. Furthermore, the parental CEM cells as well as CEM cells expressing the **Rev** wt protein were effectively killed in the course of the HIV-1 infection, whereas all CEM cells expressing the RevM10 protein were unaffected in their growth rate.

L24 ANSWER 22 OF 24 MEDLINE on STN
93018835. PubMed ID: 1402661. Stable expression of **transdominant** **Rev** protein in human T cells inhibits human immunodeficiency virus replication. Malim M H; Freimuth W W; Liu J; Boyle T J; Lyerly H K; Cullen B R; Nabel G J. (Howard Hughes Medical Institute, Ann Arbor, Michigan.) The Journal of experimental medicine, (1992 Oct 1) Vol. 176, No. 4, pp. 1197-201. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United

AB The human immunodeficiency virus (HIV) **Rev** protein is essential for viral structural protein expression (Gag, Pol, and Env) and, hence, for viral replication. In transient transfection assays, mutant forms of **Rev** have been identified that inhibit wild-type **Rev** activity and therefore suppress viral replication. To determine whether such **transdominant Rev** proteins could provide long-term protection against HIV infection without affecting T cell function, T leukemia cell lines were stably transduced with a retroviral vector encoding a **transdominant** mutant of the **Rev** protein, M10. While all the M10-expressing cell lines remained infectable by HIV-1, these same cells failed to support a productive replication cycle when infected with a cloned isolate of HIV-1. In addition, two out of three M10-expressing CEM clones were also resistant to highly productive infection by a heterogeneous HIV-1 pool. Expression of M10 did not affect induction of HIV transcription mediated by the kappa B regulatory element or **Tat**. Importantly, constitutive expression of **Rev** M10 did not alter the secretion of interleukin 2 in response to mitogen stimulation of EL-4 and Jurkat cells. The inhibition of HIV infection in cells stably expressing a **transdominant Rev** protein, in the absence of any deleterious effect on T cell function, suggests that such a strategy could provide a therapeutic effect in the T lymphocytes of acquired immunodeficiency syndrome patients.

L24 ANSWER 23 OF 24 MEDLINE on STN

91303679. PubMed ID: 2072452. Mutational definition of the human immunodeficiency virus type 1 **Rev** activation domain. Malim M H; McCarn D F; Tiley L S; Cullen B R. (Howard Hughes Medical Institute, Department of Medicine, Duke University Medical Center, Durham, North Carolina 27710.) Journal of virology, (1991 Aug) Vol. 65, No. 8, pp. 4248-54. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Replication of human immunodeficiency virus type 1 requires the functional expression of the virally encoded **Rev** protein. The binding of this nuclear trans activator to its viral target sequence, the **Rev**-response element, induces the cytoplasmic expression of unspliced viral mRNAs. Mutation of the activation domain of **Rev** generates inactive proteins with normal RNA binding capabilities that inhibit wild-type **Rev** function in a **trans-dominant** manner. Here, we report that the activation domain comprises a minimum of nine amino acids, four of which are critically spaced leucines. The preservation of this essential sequence in other primate and nonprimate lentivirus **Rev** proteins indicates that this leucine-rich motif has been highly conserved during evolution. This conclusion, taken together with the observed permissiveness of a variety of eukaryotic cell types for **Rev** function, suggests that the target for the activation domain of **Rev** is likely to be a highly conserved cellular protein(s) intrinsic to nuclear mRNA transport or splicing.

L24 ANSWER 24 OF 24 MEDLINE on STN

90311346. PubMed ID: 2195547. A **transdominant tat** mutant that inhibits **tat**-induced gene expression from the human immunodeficiency virus long terminal repeat. Pearson L; Garcia J; Wu F; Modesti N; Nelson J; Gaynor R. (Department of Medicine, University of California-Los Angeles School of Medicine 90024.) Proceedings of the National Academy of Sciences of the United States of America, (1990 Jul) Vol. 87, No. 13, pp. 5079-83. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Regulation of human immunodeficiency virus (HIV) gene expression is dependent on specific regulatory regions in the long terminal repeat. These regions include the enhancer, SP1, "TATA," and trans-activating (TAR) regions. In addition, viral regulatory proteins such as **tat** and **rev** are important in regulating HIV gene expression. The mechanism of **tat** activation remains the subject of investigation, but effects at both transcriptional and posttranscriptional levels seem likely. Previous mutagenesis of the **tat** protein revealed that the amino terminus, the cysteine-rich domain, and the basic domain were all required for complete **tat** activation. Mutants of other viral trans-acting regulatory proteins, including E1A, tax, and VM65, have been identified that were capable of antagonizing the activity of their corresponding wild-type proteins. We wished to determine whether mutants of the **tat** protein could be identified that exhibited a similar phenotype. One mutant (delta **tat**) that truncated the basic domain of **tat** resulted in a **transdominant** phenotype inhibiting **tat**-induced gene expression of the HIV long terminal repeat but not other viral promoters. This mutant exhibited its maximal phenotype in cotransfection experiments when present in an 8- to 30-fold molar excess over the wild-type **tat** gene. Trans-activation of the HIV long terminal repeat by delta **tat** was very defective at the DNA concentrations used in these experiments. RNase protection analysis indicated that this mutant decreased **tat**-induced steady-state mRNA levels of the HIV long terminal repeat. Second-site mutations of the delta **tat** gene in either the amino terminus or cysteine region eliminated the **transdominant** phenotype. In contrast to **tat**, which was localized predominantly to the nucleolus, delta **tat** was present in both the nucleus and cytoplasm, suggesting that it may inhibit

mutants of **tat** may have a role in potentially inhibiting HIV gene expression.

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     FILE 'USPATFULL' ENTERED AT 08:23:19 ON 27 JUN 2006
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                E CORDOVA C E A/IN
                E AGUILAR CORDOVA C E/IN
L1              8 S E4-E7
                E BELMONT JOHN W/IN
L2              3 S E3
L3              2 S L2 NOT L1
                E HARPER J WADE/IN
L4              1 S E3
L5              0 S L4 NOT L1

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                E BELMONT J W/IN
L7              2 S E3
L8              1 S L7 NOT L6
                E HARPER J W/IN
L9             10 S E3
L10             9 S L9 NOT L6

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     FILE 'MEDLINE' ENTERED AT 08:28:23 ON 27 JUN 2006
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L11            77 S E2-E6
L12             5 S L11 AND (TAT OR REV)
                E BELMONT J W/AU
L13           104 S E3 OR E4 OR E6
L14            99 S L13 NOT L11
L15             0 S L14 AND (TAT OR REV)
                E HARPER J W/AU
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L17           147 S L16 NOT L11
L18             1 S L17 AND (TAT OR REV)

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L21            36 S L20 AND (TAT/CLM AND REV/CLM)
L22             6 S L21 AND AY<1996

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L23           657 S (TAT AND REV)
L24            24 S L23 AND (TRANSDOMINANT OR TRANS-DOMINANT)

=> s Rev
L25          5692 REV

=> s l25 and (transdominant or trans-dominant or dominant negative or transdominant negative)
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            96000 TRANS
            93294 DOMINANT
              344 TRANS-DOMINANT
                    (TRANS(W)DOMINANT)
            93294 DOMINANT
           402725 NEGATIVE
            14653 DOMINANT NEGATIVE
                    (DOMINANT(W)NEGATIVE)
              347 TRANSDOMINANT
           402725 NEGATIVE
              122 TRANSDOMINANT NEGATIVE
                    (TRANSDOMINANT(W)NEGATIVE)
L26           118 L25 AND (TRANSDOMINANT OR TRANS-DOMINANT OR DOMINANT NEGATIVE
                  OR TRANSDOMINANT NEGATIVE)

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L28             0 L26 AND AY<1996

=> ss l26 and ay<1996
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     FILE 'USPATFULL' ENTERED AT 08:23:19 ON 27 JUN 2006
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                  E AGUILAR CORDOVA C E/IN
L1                8 S E4-E7
                  E BELMONT JOHN W/IN
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L9               10 S E3
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L11              77 S E2-E6
L12               5 S L11 AND (TAT OR REV)
                  E BELMONT J W/AU
L13             104 S E3 OR E4 OR E6
L14              99 S L13 NOT L11
L15               0 S L14 AND (TAT OR REV)
                  E HARPER J W/AU
L16             148 S E3-E5
L17             147 S L16 NOT L11
L18               1 S L17 AND (TAT OR REV)

     FILE 'USPATFULL' ENTERED AT 08:32:37 ON 27 JUN 2006
L19           13995 S (TAT AND REV)
L20             296 S L19 AND TRANSDOMINANT
L21              36 S L20 AND (TAT/CLM AND REV/CLM)
L22               6 S L21 AND AY<1996

     FILE 'MEDLINE' ENTERED AT 08:35:23 ON 27 JUN 2006
L23             657 S (TAT AND REV)
L24              24 S L23 AND (TRANSDOMINANT OR TRANS-DOMINANT)
L25            5692 S REV
L26             118 S L25 AND (TRANSDOMINANT OR TRANS-DOMINANT OR DOMINANT NEGATIVE

=> d l26,cbib,ab,100-118

L26  ANSWER 100 OF 118     MEDLINE on STN
93267767.    PubMed ID: 8388497.   Comparison of trans-dominant inhibitory
     mutant human immunodeficiency virus type 1 genes expressed by retroviral
     vectors in human T lymphocytes. Bahner I; Zhou C; Yu X J; Hao Q L;
     Guatelli J C; Kohn D B. (Division of Research Immunology/Bone Marrow
     Transplantation, Childrens Hospital Los Angeles, California. ) Journal of
     virology, (1993 Jun) Vol. 67, No. 6, pp. 3199-207.  Journal code: 0113724.
     ISSN: 0022-538X. Pub. country: United States. Language: English.
AB   trans-Dominant inhibitory mutant versions of the human
     immunodeficiency virus type 1 (HIV-1) regulatory genes tat and rev have
     previously been described.  We have constructed a series of retroviral
     vectors to transduce these genes and compare their inhibitory activities.
     The inhibitory activities were measured with transient transfection assays
     by using a reporter which expresses an HIV-1 gag-Escherichia coli lacZ
     fusion protein with strict dependence on coexpression of both tat and
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used to stably transduce human CEM T lymphocytes. The transduced CEM cells were challenged with HIV-1, and the effects of the mutant HIV-1 genes were determined by measuring the levels of HIV-1 p24gag produced. A tat gene substituted at amino acid 41 (tatk41a) retained partial trans-activating activity and lacked inhibitory activity. A tat gene with a premature stop codon at amino acid 54 (tat54ter) showed moderate **trans-dominant** inhibition of the reporter plasmid but failed to significantly inhibit HIV-1 replication. The M10 **rev** mutant, with a 2-amino-acid substitution, showed strong **trans-dominant** inhibitory activity both in the reporter plasmid and in the HIV-1 infection assay. The greatest inhibition of HIV-1 growth was seen when M10 was expressed under the transcriptional control of a human cytomegalovirus promoter; slightly less inhibition was achieved when expression of M10 was controlled by the Moloney murine leukemia virus long terminal repeat, and minimal inhibition was seen when the HIV-1 long terminal repeat controlled the M10 gene. These results demonstrate the potential utility of retroviral vectors expressing **trans-dominant** inhibitory mutant HIV-1 genes for gene therapy approaches to AIDS.

L26 ANSWER 101 OF 118 MEDLINE on STN
93233212. PubMed ID: 8474155. **Dominant negative** mutants of human T-cell leukemia virus type I Rex and human immunodeficiency virus type 1 **Rev** fail to multimerize in vivo. Bogerd H; Greene W C. (Department of Medicine, Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina 27710.) Journal of virology, (1993 May) Vol. 67, No. 5, pp. 2496-502. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Human T-cell leukemia virus type I (HTLV-I) Rex and human immunodeficiency virus type 1 (HIV-1) **Rev** are essential gene products required for the replication of these two pathogenic human retroviruses. Both Rex and **Rev** act at a posttranscriptional level by binding to highly structured RNA-response elements, the Rex-response element in HTLV-I and the **Rev**-response element in HIV-1. Using a sensitive in vivo assay of protein-protein interaction, we now demonstrate that the HTLV-I Rex and HIV-1 **Rev** proteins readily form homomultimeric complexes in the absence of their cognate RNA-response elements yet fail to form heteromultimeric complexes with each other. **Dominant negative** mutations have been identified in both the rex and **rev** genes which presumably specify a critical activation or effector domain in each of these viral transactivators. Surprisingly, these **dominant negative** mutants of Rex and **Rev** fail to interact in vivo. These findings raise the possibility that the binding of nonfunctional monomers rather than functional multimers underlies the **transdominant** phenotype of these Rex and **Rev** mutants. Further, it seems likely that the assembly of functional and stable multimers of Rex and **Rev** in vivo may depend not only on the intrinsic multimerization domains of these proteins but also on the binding of a bridging cellular cofactor to the related activation domains present in each viral transactivator.

L26 ANSWER 102 OF 118 MEDLINE on STN
93028565. PubMed ID: 1409715. Inhibition of human immunodeficiency virus type 1 replication in human T cells by retroviral-mediated gene transfer of a **dominant-negative Rev** trans-activator. Bevec D; Dobrovnik M; Hauber J; Bohnlein E. (Sandoz Research Institute, Vienna, Austria.) Proceedings of the National Academy of Sciences of the United States of America, (1992 Oct 15) Vol. 89, No. 20, pp. 9870-4. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Human immunodeficiency virus type 1 (HIV-1) is the causative agent of the acquired immunodeficiency syndrome (AIDS). Currently, no satisfactory treatment for this viral disease is available. Somatic gene therapy has been proposed as an alternative to conventional therapies. Several antiviral gene therapy approaches including ribozymes, antisense inhibition, and RNA-decoy strategies, as well as **dominant-negative** mutants of HIV-1 proteins (Gag, Tat, and **Rev**) have been suggested. To prove the concept of **trans-dominant** inhibition of HIV-1 replication, we transduced CEM cells with a retroviral vector encoding a **dominant-negative rev** gene. Amplification of integrase-specific proviral sequences from high molecular weight DNA indicated successful HIV-1 human T-lymphotropic virus type IIIB (HTLV-IIIB) infection of all cells. In contrast to CEM cells and CEM cells expressing the **rev** wild-type (wt) gene, infection of two CEM-RevM10 clones with HIV-1 did not result in the release of significant levels of p24 Gag antigen as measured by antigen capture assay, indicating a block in HIV-1 replication due to the presence of the **trans-dominant Rev** protein. Furthermore, the parental CEM cells as well as CEM cells expressing the **Rev** wt protein were effectively killed in the course of the HIV-1 infection, whereas all CEM cells expressing the RevM10 protein were unaffected in their growth rate.

L26 ANSWER 103 OF 118 MEDLINE on STN
93018835. PubMed ID: 1402661. Stable expression of **transdominant Rev** protein in human T cells inhibits human immunodeficiency virus

B R; Nabel G J. (Howard Hughes Medical Institute, Ann Arbor, Michigan.) The Journal of experimental medicine, (1992 Oct 1) Vol. 176, No. 4, pp. 1197-201. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB The human immunodeficiency virus (HIV) **Rev** protein is essential for viral structural protein expression (Gag, Pol, and Env) and, hence, for viral replication. In transient transfection assays, mutant forms of **Rev** have been identified that inhibit wild-type **Rev** activity and therefore suppress viral replication. To determine whether such **transdominant Rev** proteins could provide long-term protection against HIV infection without affecting T cell function, T leukemia cell lines were stably transduced with a retroviral vector encoding a **transdominant** mutant of the **Rev** protein, M10. While all the M10-expressing cell lines remained infectable by HIV-1, these same cells failed to support a productive replication cycle when infected with a cloned isolate of HIV-1. In addition, two out of three M10-expressing CEM clones were also resistant to highly productive infection by a heterogeneous HIV-1 pool. Expression of M10 did not affect induction of HIV transcription mediated by the kappa B regulatory element or Tat. Importantly, constitutive expression of **Rev** M10 did not alter the secretion of interleukin 2 in response to mitogen stimulation of EL-4 and Jurkat cells. The inhibition of HIV infection in cells stably expressing a **transdominant Rev** protein, in the absence of any deleterious effect on T cell function, suggests that such a strategy could provide a therapeutic effect in the T lymphocytes of acquired immunodeficiency syndrome patients.

L26 ANSWER 104 OF 118 MEDLINE on STN

92292284. PubMed ID: 1602559. **Dominant-negative** mutants are clustered in a domain of the human T-cell leukemia virus type I Rex protein: implications for trans dominance. Weichselbraun I; Berger J; Dobrovnik M; Bogerd H; Grassmann R; Greene W C; Hauber J; Bohnlein E. (SANDOZ Research Institute, Vienna, Austria.) Journal of virology, (1992 Jul) Vol. 66, No. 7, pp. 4540-5. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The 27-kDa Rex trans-acting protein appears to be essential for replication of human T-cell leukemia virus type I. Mutations introduced outside of the Rex RNA-binding domain-nucleolar localization signal display either wild-type activity or, conversely, yield **dominant-negative** proteins. We generated missense mutations in a particular domain of the Rex protein (amino acid residues 54 to 69) which is characterized by a cluster of **dominant-negative** mutants. Our results indicate that amino acids 57 to 67 are critically important for Rex function mediated through the RxRE cis-acting RNA sequence. Within this domain, only amino acids 61 to 63 could be mutated without loss of function. All other missense and deletion mutants yielded **dominant-negative** proteins. In vitro RNA-binding studies performed with glutathione S-transferase-Rex fusion proteins demonstrated that all of the mutant Rex proteins interacted specifically with RxRE RNA. Analysis of chimeric Rex-**Rev** proteins suggests that this Rex domain is important for oligomerization.

L26 ANSWER 105 OF 118 MEDLINE on STN

92194414. PubMed ID: 1548742. **trans-dominant** inhibition of human immunodeficiency virus type 1 **Rev** occurs through formation of inactive protein complexes. Hope T J; Klein N P; Elder M E; Parslow T G. (Department of Pathology, University of California, San Francisco 94143-0506.) Journal of virology, (1992 Apr) Vol. 66, No. 4, pp. 1849-55. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The human immunodeficiency virus type 1 **Rev** protein controls expression of certain viral RNAs by binding to these RNAs in the nucleus. To investigate how **dominant negative Rev** mutants inhibit **Rev** function, we fused such mutants to hormone-dependent localization signals from the glucocorticoid receptor. Each was found to have fully potent inhibitory activity whether expressed in the nucleus or in the cytoplasm. Wild-type **Rev** colocalized with an inhibitory fusion protein, implying that the two proteins interact. The resulting complexes accumulated within nuclei in response to steroids but had no effect on expression of **Rev**-responsive mRNAs. A mutation known to block in vitro oligomerization of **Rev** abolished both complex formation and inhibitory activity of the mutant fusion proteins. Thus, **trans-dominant** inhibition of **Rev** does not require competition for nuclear substrates but may instead reflect the ability of a mutant to form nonfunctional complexes with the wild-type protein in vivo.

L26 ANSWER 106 OF 118 MEDLINE on STN

91303679. PubMed ID: 2072452. Mutational definition of the human immunodeficiency virus type 1 **Rev** activation domain. Malim M H; McCarn D F; Tiley L S; Cullen B R. (Howard Hughes Medical Institute, Department of Medicine, Duke University Medical Center, Durham, North Carolina 27710.) Journal of virology, (1991 Aug) Vol. 65, No. 8, pp. 4248-54. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language:

AB Replication of human immunodeficiency virus type 1 requires the functional expression of the virally encoded **Rev** protein. The binding of this nuclear trans activator to its viral target sequence, the **Rev**-response element, induces the cytoplasmic expression of unspliced viral mRNAs. Mutation of the activation domain of **Rev** generates inactive proteins with normal RNA binding capabilities that inhibit wild-type **Rev** function in a **trans-dominant** manner. Here, we report that the activation domain comprises a minimum of nine amino acids, four of which are critically spaced leucines. The preservation of this essential sequence in other primate and nonprimate lentivirus **Rev** proteins indicates that this leucine-rich motif has been highly conserved during evolution. This conclusion, taken together with the observed permissiveness of a variety of eukaryotic cell types for **Rev** function, suggests that the target for the activation domain of **Rev** is likely to be a highly conserved cellular protein(s) intrinsic to nuclear mRNA transport or splicing.

L26 ANSWER 107 OF 118 MEDLINE on STN
91288533. PubMed ID: 1905815. The type I human T-cell leukemia virus (HTLV-I) Rex trans-activator binds directly to the HTLV-I Rex and the type 1 human immunodeficiency virus **Rev** RNA response elements. Bogerd H P; Huckaby G L; Ahmed Y F; Hanly S M; Greene W C. (Department of Medicine, Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC 27710.) Proceedings of the National Academy of Sciences of the United States of America, (1991 Jul 1) Vol. 88, No. 13, pp. 5704-8. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB The Rex protein of the type I human T-cell leukemia virus (HTLV-I) is essential for the replication of this pathogenic retrovirus and, surprisingly, can also replace the function of the structurally distinct **Rev** protein of the type 1 human immunodeficiency virus (HIV-1). Rex action requires a 255-nucleotide viral RNA stem-loop structure termed the Rex RNA response element (RexRE) located in the 3' retroviral long terminal repeat. Rex function leads to the induced cytoplasmic expression of the incompletely spliced family of viral mRNAs that uniquely encode the HTLV-I structural and enzymatic proteins (Gag, Pol, and Env). Our studies now demonstrate that Rex acts by binding directly to the RexRE in a sequence-specific manner. These effects of Rex require the presence of a 10-nucleotide subregion of the RexRE that is essential for Rex function in vivo. **Dominant-negative** mutants of Rex also bind to the RexRE with high affinity, while a recessive-negative Rex mutant altered within its arginine-rich, positively charged domain fails to engage the RexRE. Analogously, both the wild-type and **dominant-negative** Rex proteins specifically bind to the structurally distinct HIV-1 **Rev** response element, a finding that likely underlies the respective stimulatory and inhibitory effects of these HTLV-I proteins in the heterologous HIV-1 system. However, consistent with their lack of amino acid homology, the binding sites for Rex and **Rev** within the HIV-1 **Rev** response element are distinct.

L26 ANSWER 108 OF 118 MEDLINE on STN
91251242. PubMed ID: 1645796. Conserved functional organization of the human immunodeficiency virus type 1 and visna virus **Rev** proteins. Tiley L S; Malim M H; Cullen B R. (Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina 27710.) Journal of virology, (1991 Jul) Vol. 65, No. 7, pp. 3877-81. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Visna virus encodes a posttranscriptional regulatory protein that is functionally analogous to the **Rev** trans activator of human immunodeficiency virus type 1. Here, we demonstrate that the known functional organization of the human immunodeficiency virus type 1 **Rev** trans activator is shared by the distantly related visna virus **Rev** protein. In particular, both **Rev** proteins contain an N-terminal domain marked by a highly basic core motif that determines RNA sequence specificity, as well as a second C-terminal domain containing an essential leucine-rich motif that functions as an activation domain. Chimeric proteins consisting of the binding domain of one **Rev** protein fused to the activation domain of the other were fully functional in the viral sequence context cognate for the binding domain. We also describe derivatives of visna virus **Rev** bearing a defective activation domain that displayed a **trans-dominant negative** phenotype in transfected cells. These visna virus **Rev** mutants may prove useful in the derivation of transgenic animals resistant to this agriculturally important retroviral pathogen.

L26 ANSWER 109 OF 118 MEDLINE on STN
91208098. PubMed ID: 2088501. Binding of **trans-dominant** mutant **Rev** protein of human immunodeficiency virus type 1 to the cis-acting **Rev**-responsive element does not affect the fate of viral mRNA. Benko D M; Robinson R; Solomin L; Mellini M; Felber B K; Pavlakis G N. (ABL-Basic Research Program, National Cancer Institute-FCRDC, MD 21702-1201.) The New biologist, (1990 Dec) Vol. 2, No. 12, pp. 1111-22. Journal code: 9000976. ISSN: 1043-4674. Pub. country: United States. Language: English.

(HIV-1) to the cis-acting **Rev**-responsive element (RRE) was compared to the binding of a **trans-dominant Rev** mutant. RevBL, which inhibits **Rev** function. **Rev** and RevBL expressed in bacteria were purified and shown to bind in vitro to the RRE with similar affinities. The study of the RRE mutants indicated that **Rev** and RevBL bind to the same target within the RRE in vitro and in vivo. In vivo experiments demonstrated that RevBL did not increase the steady-state levels of HIV-1 mRNA or protein. These experiments suggested that additional cellular factors interacting with **Rev** but not with RevBL are necessary for function. The Rex protein of human T-cell leukemia virus type I (HTLV-I) is similar to **Rev** and acts through a sequence named Rex-responsive element (RXRE) located in the long terminal repeat of HTLV-I. We examined the function of RevBL on a hybrid mRNA molecule containing both the RRE and RXRE. While RevBL prevented **Rev** function, it did not affect Rex function on the mRNA containing either the RXRE or both the RRE and RXRE. Therefore, binding of RevBL to the RRE had neither positive nor negative effects on the mRNA, since this mRNA could be efficiently utilized in the presence of a functional Rex-RXRE interaction. The results obtained in vivo and in vitro strongly suggest that RevBL inhibits **Rev** function by binding to the same site as **Rev** and preventing **Rev** binding and function.

L26 ANSWER 110 OF 118 MEDLINE on STN
91087341. PubMed ID: 1985219. **Transdominant** repressors for human T-cell leukemia virus type I rex and human immunodeficiency virus type 1 **rev** function. Bohnlein S; Pirker F P; Hofer L; Zimmermann K; Bachmayer H; Bohnlein E; Hauber J. (SANDOZ Research Institute, Vienna, Austria.) Journal of virology, (1991 Jan) Vol. 65, No. 1, pp. 81-8. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB Human T-cell leukemia virus type I (HTLV-I) encodes a 27-kDa trans-acting gene product (Rex) which is involved in the regulated expression of transcripts coding for the viral structural proteins. We used oligonucleotide-directed mutagenesis to generate a series of mutant HTLV-I rex genes. Transient expression experiments demonstrated that 3 of 28 mutant proteins are functionally inactive on the homologous HTLV-I rex response element, whereas an additional 2 mutant proteins are functionally inactive on the heterologous human immunodeficiency virus type 1 **rev** response element. One of these mutants is able to suppress the function of the wild-type HTLV-I Rex protein in trans on the homologous rex response element sequence. Furthermore, all of these mutants are able to inhibit Rex function on the heterologous **rev** response element sequence. Intriguingly, only three of these mutants are able to inhibit the human immunodeficiency virus type 1 **Rev** protein in a **dominant-negative** manner.

L26 ANSWER 111 OF 118 MEDLINE on STN
91056570. PubMed ID: 2243384. Different sites of interaction for **Rev**, Tev, and Rex proteins within the **Rev**-responsive element of human immunodeficiency virus type 1. Solomin L; Felber B K; Pavlakis G N. (Basic Research Program, National Cancer Institute-Frederick Cancer Research and Development Center, Maryland 21702-1201.) Journal of virology, (1990 Dec) Vol. 64, No. 12, pp. 6010-7. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB We have analyzed the action of the **Rev** and Tev proteins of human immunodeficiency virus type 1 (HIV-1) and of the Rex protein of human T-cell leukemia virus type I (HTLV-I) on a series of **Rev**-responsive element (RRE) mutants. The minimum continuous RRE region necessary and sufficient for **Rev** function was determined to be 204 nucleotides. Interestingly, this region was not sufficient for Tev or Rex function. These proteins require additional sequences, which may stabilize the structure of the RRE or may contain additional sequence-specific elements. Internal RRE deletions revealed that the targets for **Rev** and Rex can be separated, since mutants responding to **Rev** and not Rex and vice versa were identified. Tev was active on both types of mutants, suggesting that it has a more relaxed specificity than do both **Rev** and Rex proteins. Although **Rev** and Rex targets within the RRE appear to be distinct, the **trans-dominant** mutant RevBL prevents the RRE interaction with Rex. RevBL cannot inhibit the function of Rex on RRE deletions that lack the **Rev**-responsive portion. These results indicate the presence of distinct sites within the RRE for interaction with these proteins. The binding sites for the different proteins do not function independently and may interfere with one another. Mutations affecting the RRE may change the accessibility and binding characteristics of the different binding sites.

L26 ANSWER 112 OF 118 MEDLINE on STN
91032987. PubMed ID: 2227413. Interaction of the human immunodeficiency virus type 1 **Rev** protein with a structured region in env mRNA is dependent on multimer formation mediated through a basic stretch of amino acids. Olsen H S; Cochrane A W; Dillon P J; Nalin C M; Rosen C A. (Department of Molecular Oncology and Virology, Roche Institute of Molecular Biology, Nutley, New Jersey.) Genes & development, (1990 Aug) Vol. 4, No. 8, pp. 1357-64. Journal code: 8711660. ISSN: 0890-9369. Pub. country: United States. Language: English.
AB Interaction of the human immunodeficiency virus type 1 (HIV-1) **Rev**

export of virus structural mRNAs from the nucleus to the cytoplasm. We show that the region encompassing the basic stretch of amino acids is essential for the ability of **Rev** to bind to RRE RNA and function in vivo. By use of a functional truncated **Rev** protein in conjunction with authentic **Rev**, effects on gel mobilities of the **Rev**-RRE RNA complex attributable to multimerization of **Rev** protein were observed. **Rev** proteins, unable to multimerize, failed to bind RRE RNA. Identification of **Rev** mutants capable of forming multimers, but unable to bind RRE RNA, suggests that the multimerization and RNA-binding domains can be distinguished and that multimerization is likely a prerequisite for formation of the RRE RNA-binding site. A mutant **Rev** protein, shown previously to function as a **trans-dominant** inhibitor of **Rev** function, bound to RRE RNA as a multimer to a similar extent as wild-type **Rev**. This observation is consistent with the hypothesis that regulation of HIV gene expression by **Rev** involves the interaction with cellular factors and that the **trans-dominant Rev** is probably defective in this function.

L26 ANSWER 113 OF 118 MEDLINE on STN
91012778. PubMed ID: 2120472. Mutational analysis of the human immunodeficiency virus type 1 **Rev** transactivator: essential residues near the amino terminus. Hope T J; McDonald D; Huang X J; Low J; Parslow T G. (Department of Pathology, University of California, San Francisco 94143-0506.) Journal of virology, (1990 Nov) Vol. 64, No. 11, pp. 5360-6. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The expression of certain mRNAs from human immunodeficiency virus type 1 (HIV-1) is controlled by the viral transactivator **Rev**, a nucleolar protein that binds a cis-acting element in these mRNAs. **Rev** is encoded by two viral exons that specify amino acids 1 to 26 and 27 to 116, respectively. Earlier studies have mapped essential regions of the protein that are encoded in the second exon. By further mutational analysis of **Rev**, we have now identified a novel locus encoded by the first exon that also is essential for transactivation in vivo. Defined by mutations at residues 14 to 20, this locus coincides with a cluster of positively charged and nonpolar amino acids that is conserved in **Rev** proteins of all known primate immunodeficiency viruses. **Rev** proteins that contained mutations at this site were defective in both nuclear localization and transactivation and did not function as **trans-dominant** inhibitors of wild-type **Rev**. Fusion of these mutants to a heterologous nuclear protein complemented the defect in localization but did not restore biological activity. Our findings suggest that this N-terminal locus may play a direct role in transactivation, perhaps contributing to essential protein-protein interactions or forming part of the RNA-binding domain of **Rev**.

L26 ANSWER 114 OF 118 MEDLINE on STN
90357785. PubMed ID: 2202148. Mutants in a conserved region near the carboxy-terminus of HIV-1 **Rev** identify functionally important residues and exhibit a **dominant negative** phenotype. Venkatesh L K; Chinnadurai G. (Institute for Molecular Virology, St. Louis University School of Medicine, Missouri 63110.) Virology, (1990 Sep) Vol. 178, No. 1, pp. 327-30. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The **rev** protein (**Rev**) of human immunodeficiency virus increases the cytoplasmic expression of viral structural gene mRNAs. We had previously reported the existence of a region (residues 73-98) near the carboxy-terminus in HIV-1 **Rev** essential for its function. To further define the structural elements in this region, we examined the effects of substitution mutations in highly conserved residues in this region, between amino acids 75-81, on **Rev** function. Mutations in Pro76-77 and Arg80 retained **Rev** function, whereas those in Leu75 and Leu81 abolished **Rev** activity and exhibited **trans-dominant** suppression of wt **Rev** function. The Leu81 mutation, in particular, exhibited an efficient **dominant negative** phenotype. Leu75 and Leu81 thus appear to define residues essential to the **Rev** "effector" function.

L26 ANSWER 115 OF 118 MEDLINE on STN
90311346. PubMed ID: 2195547. A **transdominant** tat mutant that inhibits tat-induced gene expression from the human immunodeficiency virus long terminal repeat. Pearson L; Garcia J; Wu F; Modesti N; Nelson J; Gaynor R. (Department of Medicine, University of California-Los Angeles School of Medicine 90024.) Proceedings of the National Academy of Sciences of the United States of America, (1990 Jul) Vol. 87, No. 13, pp. 5079-83. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Regulation of human immunodeficiency virus (HIV) gene expression is dependent on specific regulatory regions in the long terminal repeat. These regions include the enhancer, SP1, "TATA," and trans-activating (TAR) regions. In addition, viral regulatory proteins such as tat and **rev** are important in regulating HIV gene expression. The mechanism of tat activation remains the subject of investigation, but effects at both

mutagenesis of the tat protein revealed that the amino terminus, the cysteine-rich domain, and the basic domain were all required for complete tat activation. Mutants of other viral trans-acting regulatory proteins, including E1A, tax, and VM65, have been identified that were capable of antagonizing the activity of their corresponding wild-type proteins. We wished to determine whether mutants of the tat protein could be identified that exhibited a similar phenotype. One mutant (delta tat) that truncated the basic domain of tat resulted in a **transdominant** phenotype inhibiting tat-induced gene expression of the HIV long terminal repeat but not other viral promoters. This mutant exhibited its maximal phenotype in cotransfection experiments when present in an 8- to 30-fold molar excess over the wild-type tat gene. Trans-activation of the HIV long terminal repeat by delta tat was very defective at the DNA concentrations used in these experiments. RNase protection analysis indicated that this mutant decreased tat-induced steady-state mRNA levels of the HIV long terminal repeat. Second-site mutations of the delta tat gene in either the amino terminus or cysteine region eliminated the **transdominant** phenotype. In contrast to tat, which was localized predominantly to the nucleolus, delta tat was present in both the nucleus and cytoplasm, suggesting that it may inhibit tat function by preventing nucleolar localization. **Transdominant** mutants of tat may have a role in potentially inhibiting HIV gene expression.

L26 ANSWER 116 OF 118 MEDLINE on STN
90245647. PubMed ID: 2186373. Identification of **trans-dominant** HIV-1 **rev** protein mutants by direct transfer of bacterially produced proteins into human cells. Mermer B; Felber B K; Campbell M; Pavlakis G N. (National Cancer Institute-Frederick Cancer Research Facility, BRI-Basic Research Program, MD 21701-1013.) Nucleic acids research, (1990 Apr 25) Vol. 18, No. 8, pp. 2037-44. Journal code: 0411011. ISSN: 0305-1048. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A synthetic **rev** gene containing substitutions which introduced unique restriction sites but did not alter the deduced amino acid sequence was used as a vehicle to construct mutations in **rev**. Insertion or substitution mutations within a domain of **Rev** resulted in proteins able to inhibit the function of **Rev** protein in trans. **Rev** function was monitored in a cell line, HLfB, which contained a **rev**- mutant provirus. HLfB cells require the presence of **rev** for virus production, which was conveniently monitored by immunoblot detection of p24gag. **Trans-dominant** mutants were identified after expression in bacteria and delivery into HLfB cells by protoplast fusion. In addition, the **trans-dominant** phenotype was verified by expression of the mutant proteins in HLfB cells after cotransfection. These studies define a region between amino acid residues 81 and 88 of **rev**, in which different mutations result in proteins capable of inhibiting **Rev** function.

L26 ANSWER 117 OF 118 MEDLINE on STN
90003221. PubMed ID: 2676192. HIV-1 Gag mutants can dominantly interfere with the replication of the wild-type virus. Trono D; Feinberg M B; Baltimore D. (Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142.) Cell, (1989 Oct 6) Vol. 59, No. 1, pp. 113-20. Journal code: 0413066. ISSN: 0092-8674. Pub. country: United States. Language: English.

AB The products of the human immunodeficiency virus (HIV) gag gene exist in a highly multimerized state in the mature virion. For that reason, they may represent a particularly suitable target for the generation of **dominant negative** mutants. A number of HIV site-directed Gag mutants did show interference with the production of infectious viral particles from cells in which they were cotransfected with a wild-type proviral DNA. Furthermore, cells constitutively expressing such HIV Gag mutants had an impaired ability to support HIV replication when infected with wild-type virus. The block was localized to the late stages of the virus life cycle. Such Gag variants could constitute prototypes for the development of anti-HIV intracellular immunization.

L26 ANSWER 118 OF 118 MEDLINE on STN
89324054. PubMed ID: 2752419. Functional dissection of the HIV-1 **Rev** trans-activator--derivation of a **trans-dominant** repressor of **Rev** function. Malim M H; Bohnlein S; Hauber J; Cullen B R. (Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina 27710.) Cell, (1989 Jul 14) Vol. 58, No. 1, pp. 205-14. Journal code: 0413066. ISSN: 0092-8674. Pub. country: United States. Language: English.

AB Human immunodeficiency virus type 1 (HIV-1) encodes a nuclear trans-activator, termed **Rev**, that is required for the expression of the viral structural proteins and, hence, for viral replication. The **Rev** protein acts posttranscriptionally to induce the sequence-specific nuclear export of unspliced HIV-1 mRNA species that are otherwise excluded from the cell cytoplasm. We have used site-directed mutagenesis to identify two distinct regions of the HIV-1 **Rev** protein that are required for in vivo biological activity. The larger and more N-terminal of these two regions includes, but extends beyond, an arginine-rich sequence element required for nuclear localization. Mutation of a second, more C-terminal

that act as **trans-dominant** inhibitors of **Rev** function. These **Rev** mutants are shown to inhibit HIV-1 replication when expressed in transfected cells and may have potential application in the treatment of HIV-1 related disease.

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L26 ANSWER 75 OF 118 MEDLINE on STN
96256811. PubMed ID: 8676525. Inhibition of human immunodeficiency virus type 1 replication is enhanced by a combination of **transdominant** Tat and **Rev** proteins. Ulich C; Harrich D; Estes P; Gaynor R B. (Division of Molecular Virology, Department of Medicine, University of Texas Southwestern Medical Center at Dallas, Texas 75235-8594, USA.) Journal of virology, (1996 Jul) Vol. 70, No. 7, pp. 4871-6. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB Mutation of either of two critical human immunodeficiency virus type 1 (HIV-1) regulatory proteins, Tat and **Rev**, results in marked defects in viral replication. Thus, inhibition of the function of one or both of these proteins can significantly inhibit viral growth. In the present study, we constructed a novel **transdominant** Tat mutant protein and compared its efficiency in inhibiting HIV-1 replication with that of **transdominant** mutant **Rev** M10 when these proteins were stably expressed either alone or in combination in T-lymphocyte cell lines. The **transdominant** Tat mutant protein alone resulted in a modest inhibition of HIV replication, but it was able to enhance the ability of the M10 **Rev** mutant protein to inhibit HIV-1 replication. These results suggest a possible synergistic effect of these **transdominant** mutant proteins in inhibiting HIV-1 replication.

L26 ANSWER 76 OF 118 MEDLINE on STN
96238293. PubMed ID: 8787346. [Gene therapy for hereditary and acquired human diseases]. Therapie genique de maladies humaines hereditaires et acquises. Mehtali M; Imler J L; Sorg T; Pavirani A. (TRANSGENE S.A., Strasbourg.) Annales d'endocrinologie, (1995) Vol. 56, No. 6, pp. 571-4. Ref: 15. Journal code: 0116744. ISSN: 0003-4266. Pub. country: France. Language: French.
AB Cystic Fibrosis (CF) and AIDS are primary candidate disorders to be treated by gene therapy, owing to their lethality and the absence of efficient clinical treatments. Treatment of CF by gene therapy will require the transfer of the functional CFTR cDNA into the diseased human airway epithelia since mutations within the CFTR gene are responsible for CF. We have therefore cloned the human CFTR cDNA and developed a recombinant E1-deleted adenoviral vector carrying a CFTR expression cassette. We demonstrated in vitro the ability of this vector to efficiently transduce human lung cells isolated from CF patients and to correct their phenotype. Efficient in, vivo delivery of the CFTR cDNA to the airways of cotton rats and rhesus monkeys was also obtained and no dissemination of the recombinant viral vector in other tissues than the airways was observed. We have therefore designed a phase I clinical trial involving CF patients. In contrast to the monogenic CF disease, the mechanisms of AIDS pathogenesis still remain poorly understood. Such limited knowledge of the disease constitutes a serious restriction to the development of a rational gene therapy strategy for AIDS. Since HIV, the causative agent of AIDS, predominantly infects cells of the hematopoietic system, pluri- or multipotent stem cells may constitute potential targets for the introduction of a foreign anti-HIV gene that will inhibit HIV replication and/or spread. Reimplantation of the genetically modified stem cells into asymptomatic HIV-infected patients should theoretically allow the repopulation of the host's immune system with mature CD4+ cells expressing novel molecules that interfere with viral replication, thus slowing the progression of AIDS. We identified several new **transdominant** inhibitors derived from the viral TAT and **REV** proteins and showed their ability to confer to human CD4 lymphocytes resistance against HIV1 infection. Retroviral vectors carrying these potential therapeutic genes have been developed and are currently being tested in vivo in newly developed transgenic animal models, in humanized SCID mice and in macaques.

L26 ANSWER 77 OF 118 MEDLINE on STN
96159131. PubMed ID: 8573391. Analysis of **trans-dominant** mutants of the HIV type 1 **Rev** protein for their ability to inhibit **Rev** function, HIV type 1 replication, and their use as anti-HIV gene therapeutics. Ragheb J A; Bressler P; Daucher M; Chiang L; Chuah M K; Vandendriessche T; Morgan R A. (National Heart, Lung, and Blood Institute, Bethesda, Maryland, USA.) AIDS research and human retroviruses, (1995 Nov) Vol. 11, No. 11, pp. 1343-53. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.
AB The HIV-1 **rev** gene product facilitates the transport of singly spliced and unspliced HIV-1 transcripts and is necessary for productive HIV-1 infection. On the basis of the previously described **trans-dominant** **Rev** mutant M10, four point mutants and one frameshift mutant of the

expression vectors and analyzed for their ability to inhibit **Rev**-mediated gene expression. Transient transfection systems were used to screen these new mutants, and each was shown to inhibit expression of a **Rev**-dependent CAT reporter plasmid. Inhibition of HIV-1 envelope gene expression was tested in the HeLa-T4 cell line and was also shown to be inhibited by the **trans-dominant Rev** mutants. Retroviral vector producer cell lines were constructed and used to transduce **Rev trans-dominant** genes into the human T-cell line SupT1. The engineered SupT1 cell lines were then challenged with HIV-1 IIIB and HIV-1 expression was monitored by Northern blot analysis and in situ hybridization. SupT1 cells expressing either a **Rev** point mutant or the frameshift mutant showed greatly reduced HIV-1 mRNA accumulation and the **Rev**-dependent singly spliced and unspliced HIV-1 mRNAs were reduced. The kinetics of viral replication following challenge of **Rev trans-dominant**-engineered SupT1 cells with both HIV-1 IIIB and MN strains was significantly reduced and cells were protected from viral lysis. Viruses that emerge late in infection from **Rev trans-dominant**-engineered cultures are not resistant to **Rev**-mediated inhibition. Last, **trans-dominant Rev**-mediated protection of human CD4+ lymphocytes from challenge with primary HIV-1 patient isolates confirms the potential utility of this system as an anti-HIV-1 gene therapy approach.

L26 ANSWER 78 OF 118 MEDLINE on STN
96144820. PubMed ID: 8551047. Detection of intracellular HIV-1 **Rev** protein by flow cytometry. Rigg R J; Dando J S; Escaich S; Plavec I; Bohnlein E. (Progenesys, Palo Alto, CA 94304, USA.) Journal of immunological methods, (1995 Dec 27) Vol. 188, No. 2, pp. 187-95. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB The **Rev** trans-activator protein plays a pivotal role in human immunodeficiency virus type 1 (HIV-1) replication by allowing expression of the viral structural proteins. We have developed a protocol to quantitatively assay intracellular steady state levels of **Rev** Ag (**Rev** wild type and RevM10 proteins) by flow cytometry. Three fixation and permeabilization techniques were compared. These protocols varied in the magnitude of the signal which could be detected, and in the ability to distinguish between **Rev** Ag positive and negative populations. This technology is applicable to a variety transduced or transfected cell types (species, lineage), and for cell lines and primary cells acutely infected with HIV-1. The assay is therefore a valuable tool both to analyze **Rev** protein expression levels in HIV-infected cells and to optimize delivery of the **dominant-negative** RevM10 gene for clinical gene therapy applications. In addition, a second, independent intracellular protein (HIV-Tat) has been detected using the same approach.

L26 ANSWER 79 OF 118 MEDLINE on STN
96078097. PubMed ID: 7578399. RevM10-mediated inhibition of HIV-1 replication in chronically infected T cells. Escaich S; Kalfoglou C; Plavec I; Kaushal S; Mosca J D; Bohnlein E. (Progenesys, Palo Alto, CA 94304, USA.) Human gene therapy, (1995 May) Vol. 6, No. 5, pp. 625-34. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB Two clinical regimens have been proposed for gene therapies of acquired immunodeficiency syndrome (AIDS): (i) Genetic modification of differentiated peripheral mononuclear cells ex vivo and (ii) gene delivery into hematopoietic stem/progenitor cells ex vivo. Various antiviral strategies targeted at different molecular processes in the human immunodeficiency virus type 1 (HIV-1) life cycle are currently being pursued, all with the goal of reducing HIV-1 replication. Until now, all successful studies have reported inhibition in acutely HIV-infected cells that had been genetically modified prior to infection. These promising results do not address a clinically relevant question: What is the contribution of already infected peripheral mononuclear and hematopoietic stem/progenitor cells to disease progression? In this report, we demonstrate inhibition of both HIV-1 replication and production of infectious particles in chronically infected human T leukemia cell lines. The antiviral effect on the transduced cell population correlates with the expression of the **dominant-negative** RevM10 protein. This is the first demonstration that a gene therapy-based treatment can achieve antiviral efficacy in human T leukemia cells chronically infected with HIV-1.

L26 ANSWER 80 OF 118 MEDLINE on STN
96074519. PubMed ID: 7491768. Analysis of trafficking of **Rev** and **transdominant Rev** proteins in living cells using green fluorescent protein fusions: **transdominant Rev** blocks the export of **Rev** from the nucleus to the cytoplasm. Stauber R; Gaitanaris G A; Pavlakis G N. (Human Retrovirus Section, NCI-FCRDC, Maryland 21702-1201, USA.) Virology, (1995 Nov 10) Vol. 213, No. 2, pp. 439-49. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Expression of gag/pol and env genes of human immunodeficiency virus requires the viral **Rev** protein. Mutant **Rev** proteins, displaying a **transdominant** phenotype (TDRev), were shown to inhibit **Rev** function.

fluorescent protein (GFP) of Aequorea victoria was fused to **Rev** and TDRev, which allowed the study of their trafficking and interactions in living human cells. Both **Rev**-GFP and TDRev-GFP were shown to retain appropriate nucleolar localization and function. Upon actinomycin D treatment, **Rev**-GFP was transported to the cytoplasm within 1.5 hr, while TDRev, although partially dissociated from the nucleolus, was retained in the nucleus. Coexpression of **Rev**-GFP and TDRev in the same cell demonstrated that TDRev inhibited the transport of **Rev**-GFP from the nucleus to the cytoplasm. This inhibition was specific for **Rev**, since the export of the functionally analogous Rex protein of the human T-cell leukemia virus type I was not inhibited by TDRev. These results indicate that **Rev** and TDRev form heteromultimers in the nucleolus and that this interaction prevents **Rev**'s export from the nucleus to the cytoplasm. In addition to providing a model for the function of TDRev, these results also demonstrate the successful application of protein fusions to GFP to study localization and trafficking of proteins in living mammalian cells.

L26 ANSWER 81 OF 118 MEDLINE on STN
96050917. PubMed ID: 7584057. Regulated expression of a **dominant negative** form of **Rev** improves resistance to HIV replication in T cells. Liu J; Woffendin C; Yang Z Y; Nabel G J. (Howard Hughes Medical Institute, University of Michigan Medical Center, Department of Internal Medicine, Ann Arbor 48109-0650, USA.) Gene therapy, (1994 Jan) Vol. 1, No. 1, pp. 32-7. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Infection by the human immunodeficiency virus (HIV) has remained refractory to treatment, and molecular genetic interventions have been developed for the treatment of the acquired immunodeficiency syndrome (AIDS). Previous studies have focused on the development of gene products which inhibit productive HIV replication, including **transdominant** proteins, RNA decoys and ribozymes. In this report, we show that appropriate expression vectors which optimize production and regulated synthesis of a **transdominant** mutant form of **Rev** improve its antiviral effect. The combination of a strong constitutive enhancer, a Tat activation response (TAR) regulatory element and **transdominant Rev** take advantage of three aspects of early viral gene expression to confer increased resistance to HIV replication. This vector may be useful, alone or in combination with other antiviral genes, to provide gene therapy for AIDS.

L26 ANSWER 82 OF 118 MEDLINE on STN
96022631. PubMed ID: 7578421. Genetic modification of human peripheral blood lymphocytes with a **transdominant negative** form of **Rev**: safety and toxicity. Fox B A; Woffendin C; Yang Z Y; San H; Ranga U; Gordon D; Osterholzer J; Nabel G J. (Howard Hughes Medical Institute, University of Michigan Medical Center, Ann Arbor 48109-0650, USA.) Human gene therapy, (1995 Aug) Vol. 6, No. 8, pp. 997-1004. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB A **transdominant** mutant form of the **rev** gene, M10, confers resistance to infection by the human immunodeficiency virus (HIV) in vitro and is currently under investigation as a potential intervention in acquired immunodeficiency syndrome (AIDS). In this report, we examine three issues relevant to the safety of autologous transfer of human T cells genetically modified with **Rev** M10. First, the potential for malignant transformation was assessed in vitro using interleukin-2 (IL-2) dependence and fibroblast transformation assays, and tumorigenicity was evaluated in severe combined immunodeficient (SCID) mice. Possible toxicity was evaluated by pathologic analysis following adoptive transfer of genetically modified human T cells into SCID mice. Second, methods were developed that permit T cell activation required for gene transfer but do not allow replication of endogenous HIV. Third, T cell function was evaluated in peripheral blood lymphocytes (PBL) of HIV-seropositive donors transduced with **Rev** M10 and compared to a negative control mutant, delta **Rev** M10. By all criteria, no oncogenicity or toxicity was observed. Human T cells transduced with these vectors did not grow in the absence of IL-2 in vitro, and no tumors were observed following transplantation of genetically modified human cells into recipient SCID mice. Histopathological analysis of heart, lung, liver, spleen, and kidney of animals 1-21 weeks following adoptive transfer of gene-modified human T cells revealed no significant abnormalities. Additionally, no differences were observed in the pattern of cytokine secretion in enriched human PBL expressing **Rev** M10 compared to delta **Rev** M10. (ABSTRACT TRUNCATED AT 250 WORDS)

L26 ANSWER 83 OF 118 MEDLINE on STN
96022225. PubMed ID: 7475320. Gene therapy against retroviral diseases. Essex M; Matsuda Z; Yu X; Lee T H. (Department of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts 02115, USA.) Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K, (1995 Oct) Vol. 9 Suppl 1, pp. S71-4. Ref: 13. Journal code: 8704895. ISSN: 0887-6924. Pub. country: ENGLAND: United Kingdom. Language: English.

infections, including retroviral infections. Human retroviral diseases fit two categories: (1) those that result from a monoclonal outgrowth of a human T-cell leukemia virus type I (HTLV-I)-infected cell, as in the case of adult T cell leukemia (ATL); and (2) those that appear to result directly from virus load rather than monoclonal outgrowth--such as tropical spastic paraparesis/HTLV-I associated myelopathy (TSP/HAM) and human immunodeficiency virus (HIV)-associated acquired immune deficiency syndrome (AIDS). For ATL gene therapy, corrective mechanisms directed at regulatory sequences rather than viral sequences may be most important, though perhaps anti-tax therapy would be useful. For TSP/HAM and AIDS, gene therapy directed to control virus replication may be most useful. For anti-retroviral therapy, one may use **dominant negative** mutants and a variety of other approaches that direct toxins or compete out viral regulatory gene signal sequences. For maximum benefit, such therapy should be directed to different essential genes (eg gag, pol, env, tat or **rev**) involved in the virus replication cycle and utilize different toxic approaches. A major impediment to the use of gene therapy for AIDS is our inability to transfect a significant fraction of target cells in vivo. Except for reconstituted mice, retroviral systems of animals have been under-utilized as models for gene therapy. Naturally occurring retroviral diseases of cats, goats, horses, and other species provide models for future development.

L26 ANSWER 84 OF 118 MEDLINE on STN
95373148. PubMed ID: 7645223. The activation domain of simian immunodeficiency virus SIVmac239 **Rev** protein is structurally and functionally analogous to the HIV-1 **Rev** activation domain. Berchtold S; Hornung U; Aepinus C. (Institut fur Klinische und Molekulare Virologie, Universitat Erlangen-Nurnberg, Federal Republic of Germany.) Virology, (1995 Aug 1) Vol. 211, No. 1, pp. 285-9. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The **Rev** proteins of primate immunodeficiency viruses are essential transactivators for the switch from early to late phase in the viral replication cycle. By mutational analysis, a putative activation domain (AD) has been assigned to the carboxy-terminus. This leucine-rich stretch of amino acids proved to be essential for the transactivating properties of HIV-1 **Rev**. Some mutants in the AD transdominantly inhibit the function of wild-type **Rev** protein very efficiently. We identified a similar domain structure for SIVmac239 **Rev** by sequence comparison and in vitro mutagenesis. The leucine/isoleucine residues of the SIVmac239 **Rev** activation domain appeared to be of similar importance for function. The mutants of these residues in the SIV AD displayed a **dominant negative** phenotype on both HIV-1 and SIVmac 239 **rev**-responsive elements (RRE). The prokaryotically expressed wild-type and mutant proteins were analyzed for RNA-binding properties in a gel-shift assay in vitro. This assay revealed a similar binding pattern of wild-type and **transdominant** proteins on either RRE.

L26 ANSWER 85 OF 118 MEDLINE on STN
95339003. PubMed ID: 7614248. Inhibition of HIV-1 by a double **transdominant** fusion gene. Aguilar-Cordova E; Chinen J; Donehower L A; Harper J W; Rice A P; Butel J S; Belmont J W. (Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030, USA.) Gene therapy, (1995 May) Vol. 2, No. 3, pp. 181-6. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A double **transdominant** fusion gene (trev) designed to inhibit two essential HIV functions simultaneously was constructed by linking tat and **rev transdominant** mutants. Trev independently inhibited both Tat and **Rev** functions, localized within the nucleus and cells transfected with trev showed a stable inhibition of HIV-1-mediated cytopathicity. A retroviral vector of trev was made and shown also to confer protection from HIV cytopathic effects. Simultaneous inhibition of two essential viral genes presents significant advantages for potential gene therapy treatment of HIV infection over conventional single effect molecules.

L26 ANSWER 86 OF 118 MEDLINE on STN
95333304. PubMed ID: 7609088. Suppression of simian immunodeficiency virus replication by human immunodeficiency virus type 1 **trans-dominant negative rev** mutants. Endres C L; Bergquam E P; Axthelm M K; Wong S W. (Division of Pathobiology and Immunology, Oregon Regional Primate Research Center, Beaverton 97006, USA.) Journal of virology, (1995 Aug) Vol. 69, No. 8, pp. 5164-6. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We demonstrate that **trans-dominant negative rev** mutants are able to suppress simian immunodeficiency virus provirus replication in both transient cotransfection assays and stably transduced HUT 78 cells. These studies suggest that the efficacy of **trans-dominant rev** strategies in reducing viral burden may be evaluated in a simian immunodeficiency virus-rhesus macaque animal model.

L26 ANSWER 87 OF 118 MEDLINE on STN
95287453. PubMed ID: 7769662. Inhibition of clinical human

by retroviral vectors expressing anti-HIV genes. Vandendriessche T; Chuah M K; Chiang L; Chang H K; Ensoli B; Morgan R A. (Clinical Gene Therapy Branch, National Cancer Institute, Bethesda, Maryland 20892, USA.) Journal of virology, (1995 Jul) Vol. 69, No. 7, pp. 4045-52. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Gene therapy may be of benefit in human immunodeficiency virus type 1 (HIV-1)-infected individuals by virtue of its ability to inhibit virus replication and prevent viral gene expression. It is not known whether anti-HIV-1 gene therapy strategies based on antisense or **transdominant** HIV-1 mutant proteins can inhibit the replication and expression of clinical HIV-1 isolates in primary CD4+ T lymphocytes. We therefore transduced CD4+ T lymphocytes from uninfected individuals with retroviral vectors expressing either HIV-1-specific antisense-TAR or antisense-Tat/**Rev** RNA, **transdominant** HIV-1 **Rev** protein, and a combination of antisense-TAR and **transdominant Rev**. The engineered CD4+ T lymphocytes were then infected with four different clinical HIV-1 isolates. We found that replication of all HIV-1 isolates was inhibited by all the anti-HIV vectors tested. Greater inhibition of HIV-1 was observed with **transdominant Rev** than with antisense RNA. We hereby demonstrated effective protection by antisense RNA or **transdominant** mutant proteins against HIV-1 infection in primary CD4+ T lymphocytes using clinical HIV-1 isolates, and this represents an essential step toward clinical anti-HIV-1 gene therapy.

L26 ANSWER 88 OF 118 MEDLINE on STN

95264419. PubMed ID: 7745679. Nuclear export of the human immunodeficiency virus type 1 nucleocytoplasmic shuttle protein **Rev** is mediated by its activation domain and is blocked by **transdominant negative** mutants. Szilvay A M; Brokstad K A; Kopperud R; Haukenes G; Kalland K H. (National Centre for Research in Virology, Gade Institute, University of Bergen, Norway.) Journal of virology, (1995 Jun) Vol. 69, No. 6, pp. 3315-23. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The human immunodeficiency virus type 1 nucleocytoplasmic shuttle protein **Rev** moves repeatedly between the cytoplasm, a perinuclear zone, the nucleoli, and nucleoplasmic speckles. In this study, we demonstrated by both indirect immunofluorescence and Western immunoblot analysis that nuclear exit of **Rev transdominant negative** mutants was defective compared with that of wild-type **Rev**. The basic and activation domains of **Rev** signal import and export, respectively, of **Rev** across the nuclear membrane. In cotransfection experiments, mutants containing mutations of **Rev** inhibited the nuclear egress of wild-type **Rev**, thus revealing a novel **transdominant negative** phenotype.

L26 ANSWER 89 OF 118 MEDLINE on STN

95172149. PubMed ID: 7867718. Nucleocytoplasmic transport of the **Rev** protein of human immunodeficiency virus type 1 is dependent on the activation domain of the protein. Wolff B; Cohen G; Hauber J; Meshcheryakova D; Rabeck C. (Sandoz Research Institute, Vienna, Austria.) Experimental cell research, (1995 Mar) Vol. 217, No. 1, pp. 31-41. Journal code: 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB The human immunodeficiency virus type 1 (HIV-1) regulatory protein **Rev**, which is required for the cytoplasmic expression of unspliced and incompletely spliced viral mRNAs, is located predominantly in the nucleolus. In this study, we show that **Rev** translocates from the nucleolus to the cytoplasm in HeLa and COS cells transfected with **Rev** under conditions where rRNA synthesis is inhibited (e.g., with actinomycin D). **Dominant-negative** mutants with mutations in the activation domain of **Rev**, which are known to inhibit wild-type **Rev** function in trans, are unable to leave the nucleus upon actinomycin D treatment. More importantly, when present in excess, these mutants inhibit the translocation of wild-type **Rev**. This correlation of inhibitory activities suggests that **Rev** function depends on its transport to and presence (at least transient) in the cytoplasm. In this context, we discuss the possibility that **Rev** is actively involved in the transport of HIV-1-specific mRNAs containing the **Rev** response element (a highly structured RNA sequence, which is specifically recognized by the **Rev** trans-activator). We also discuss the potential of nucleocytoplasmic export of **Rev** as a target for anti-HIV chemotherapy.

L26 ANSWER 90 OF 118 MEDLINE on STN

95169467. PubMed ID: 7865286. Effect of mutations in **rev** gene of SIVmac on virus replication. Aavik E; Hakkarainen K; Krohn K J. (Institute of Biomedical Sciences, University of Tampere, Finland.) AIDS research and human retroviruses, (1994) Vol. 10 Suppl 2, pp. S123-8. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB The functional activity of SIVmac251 **Rev** was altered by introducing amino acid changes inside and chain termination mutations after the **Rev** response element-binding region (RBR) of the protein. The effects of specific mutations were evaluated by transfecting proviral DNAs into the

Rev or HTLV-1 Rex proteins. Cell-free supernatants from these transient expression assays were further characterized by infecting CD4-positive lymphoid cell lines H9 and MT-4, the latter abortively infected with HTLV-1, and human peripheral blood mononuclear cells. These results together with the data from cotransfection experiments show that SIV can be attenuated up to 95% by introducing changes into the arginine-rich domain, RBR, of **Rev**. These recessive mutations were efficiently complemented in trans by HIV-1 **Rev**, SIV **Rev**, and HTLV-I Rex proteins. In contrast, the mutants of **Rev** protein that had a chain termination after RBR were **trans-dominant negative** and could not be trans-complemented with any of these three regulatory proteins. When additional mutations were inserted into the RBR of these trans-dominantly negative **Rev** proteins, complementation was obtained again.

L26 ANSWER 91 OF 118 MEDLINE on STN
95156585. PubMed ID: 7853493. Use of a human immunodeficiency virus type 1 **Rev** mutant without nucleolar dysfunction as a candidate for potential AIDS therapy. Furuta R A; Kubota S; Maki M; Miyazaki Y; Hattori T; Hatanaka M. (Department of Molecular Virology, Kyoto University, Japan.) Journal of virology, (1995 Mar) Vol. 69, No. 3, pp. 1591-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Applications of **transdominant** mutants of human immunodeficiency virus type 1 (HIV-1) regulatory proteins, especially **Rev** mutant, have been attempted for gene therapy against AIDS, because the **Rev** protein is essential for viral replication. We have previously reported that a mutant **Rev** protein (dRev) lacking its nucleolar targeting signal remained out of nuclei in expressed cells and strongly inhibited the function of **Rev**. To investigate the effects of dRev on HIV-1 replication, we established several dRev-expressing human cell lines with two different vector systems and examined virus production in these cells. An HIV-1-derived vector containing drev cDNA was constructed and introduced into CD4-positive HeLa cells and cells of the human T-cell line CCRF-CEM (CEM). In dRev-expressing HeLa cells, virus replication, syncytium formation, and cell death caused by HIV-1 infection were remarkably suppressed, and the same vector also conferred a resistant phenotype on CEM cells. The production was also suppressed in CEM cells containing the drev gene driven by a cytomegalovirus promoter. In addition, we found that dRev did not cause nucleolar dysfunction in a transient assay, in contrast to other **transdominant** mutants and wild-type **Rev**. Since dRev cannot migrate into the nuclei, it is expected not to interfere with nuclear/nucleolar functions of the host cell. We conclude that dRev is one promising candidate as an antiviral molecule for gene therapy against AIDS.

L26 ANSWER 92 OF 118 MEDLINE on STN
95062312. PubMed ID: 7972106. Nonviral and viral delivery of a human immunodeficiency virus protective gene into primary human T cells. Woffendin C; Yang Z Y; Udaykumar; Xu L; Yang N S; Sheehy M J; Nabel G J. (Howard Hughes Medical Institute, University of Michigan Medical Center, Ann Arbor.) Proceedings of the National Academy of Sciences of the United States of America, (1994 Nov 22) Vol. 91, No. 24, pp. 11581-5. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Because AIDS has been refractory to traditional pharmacologic interventions, alternative approaches have been developed. Although the introduction of specific antiviral genes into T leukemia cells can provide relative resistance to human immunodeficiency virus (HIV) replication, the testing of such genes against primary viral isolates in human CD4+ lymphocytes has been limited, and safety questions remain regarding gene delivery into cells from HIV-infected patients. In this report, we evaluate the efficacy of a **transdominant** mutant protein, **Rev** M10, against cloned and primary HIV isolates in human peripheral blood lymphocytes and describe different methods of gene transfer into peripheral blood lymphocytes from HIV-infected individuals. We show that gold microparticles can mediate stable **Rev** M10 gene transfer into these cells. Introduction of **Rev** M10 by these techniques conferred resistance to HIV infection in vitro to cloned and clinical isolates. Nonviral delivery of HIV protective genes will facilitate the development of gene therapy for AIDS and the analysis of viral and cellular gene expression in human T lymphocytes.

L26 ANSWER 93 OF 118 MEDLINE on STN
94378522. PubMed ID: 8091675. Exchange of functional domains between **Rev** proteins of HIV-1 and SIVmac239 results in a **dominant negative** phenotype. Berchtold S; Ries J; Hornung U; Aepinus C. (Institut fur klinische und molekulare Virologie, Universitat Erlangen-Nurmberg, Federal Republic of Germany.) Virology, (1994 Oct) Vol. 204, No. 1, pp. 436-41. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The **Rev** proteins of primate immunodeficiency viruses are essential transactivators to switch from early to late phase in the viral replication cycle. Surprisingly, the **Rev** protein of HIV-1 is able to

vice versa. To understand the underlying mechanism of this incomplete functional reciprocity, we constructed a series of chimeric HIV-1/SIVmac239 **Rev** proteins and tested for transcomplementation efficacy on **Rev**-dependent indicator plasmids. In addition, we analyzed the prokaryotically expressed wild type and chimeric proteins for RNA-binding properties in a gel-shift assay in vitro. The functional defect of SIVmac239 on the HIV-1 **Rev** response element (RRE) is not due to a lack of binding or multimerization. In cotransfection experiments, SIVmac239 **Rev** and the chimeric proteins were tested for potential inhibitory effects on HIV-1 **Rev** function using the HIV-1 based indicator plasmid. Some of these proteins turned out to be **transdominant** inhibitors almost as potent as the HIV-1 **Rev** mutant M10 which is localized in the activation domain and is one of the strongest **transdominant** inhibitors. Surprisingly, M10 was not able to inhibit the function of either **Rev** protein on SIVmac239 RRE, whereas a corresponding SIVmac239 **Rev** mutant (SIV M10) was a **transdominant** inhibitor of SIVmac239 **Rev** function on its homologous RRE.

L26 ANSWER 94 OF 118 MEDLINE on STN
94224800. PubMed ID: 8170964. A **Rev**-inducible mutant gag gene stably transferred into T lymphocytes: an approach to gene therapy against human immunodeficiency virus type 1 infection. Smythe J A; Sun D; Thomson M; Markham P D; Reitz M S Jr; Gallo R C; Lisziewicz J. (Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.) Proceedings of the National Academy of Sciences of the United States of America, (1994 Apr 26) Vol. 91, No. 9, pp. 3657-61. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB One strategy for somatic gene therapy for human immunodeficiency virus type 1 (HIV-1) infection is based on the regulated expression of **dominant negative** mutants of the HIV-1 gag gene. To limit expression of the mutant Gag polypeptide to HIV-1-infected cells, we have constructed a replication-defective retroviral vector that contains a **Rev**-responsive element. By using this construct we have obviated problems that can be associated with constitutive expression of an exogenous gene, an important step toward developing a human therapy. In uncloned T lymphocytes infected (transduced) with this retroviral construct, HIV-1 replication was inhibited by 94% with a concomitant decrease in the cytopathic effects of the virus. In addition, simian immunodeficiency virus (SIV) replication was also shown to be significantly inhibited, suggesting that this mutant Gag protein may have antiviral efficacy against a broad range of primate lentiviruses and that an SIV/macaque model can be used for further in vivo studies. These results have important implications in assessing the potential of somatic gene therapy in the treatment of HIV-1 infection.

L26 ANSWER 95 OF 118 MEDLINE on STN
94207076. PubMed ID: 8155773. A molecular genetic intervention for AIDS--effects of a **transdominant negative** form of **Rev**. Nabel G J; Fox B A; Post L; Thompson C B; Woffendin C. (University of Michigan Medical Center.) Human gene therapy, (1994 Jan) Vol. 5, No. 1, pp. 79-92. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

L26 ANSWER 96 OF 118 MEDLINE on STN
94107961. PubMed ID: 8280800. The development and testing of retroviral vectors expressing **trans-dominant** mutants of HIV-1 proteins to confer anti-HIV-1 resistance. Liem S E; Ramezani A; Li X; Joshi S. (University of Toronto, Department of Microbiology, Ontario, Canada.) Human gene therapy, (1993 Oct) Vol. 4, No. 5, pp. 625-34. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB **Trans-dominant** mutants of human immunodeficiency virus type 1 (HIV-1) Tat and **Rev** are attractive candidates for use in gene therapy in the treatment of HIV-1 infections because both are essential for viral replication. Retroviral vectors were constructed to allow either Tat-inducible or Tat- and **Rev**-inducible expression of **trans-dominant** mutants of Tat and **Rev**. These vectors were used to infect a human CD4+ lymphocyte-derived cell line, MT4. To determine the efficacy of various Tat and **Rev** mutants in inhibiting HIV-1 multiplication, MT4 cells containing mutant-expressing constructs were infected with HIV-1, and the amount of HIV-1 released in the culture medium was measured for up to 30 days. A high level of resistance was observed in cells expressing the double tat/**rev** mutant in a Tat-inducible manner.

L26 ANSWER 97 OF 118 MEDLINE on STN
94079809. PubMed ID: 8257633. Human immunodeficiency virus type 2 (HIV-2) trans-activator (Tat): functional domains and the search for **trans-dominant negative** mutants. Arya S K. (Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.) AIDS research and human retroviruses, (1993 Sep) Vol. 9, No. 9, pp. 839-48. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

important trans-regulator of viral gene expression. It differs from the related HIV-1 Tat in certain aspects of its structure and function. HIV-2 Tat is composed of 130 amino acids versus 86 amino acids for HIV-1 Tat. Apart from certain conserved regions, there is little homology between the two Tats. They also differ in their ability to trans-activate HIV-2 and HIV-1 long terminal repeat (LTR)-directed gene expression. As an aid to understanding its mechanism of action, the functional domains important for HIV-2 Tat trans-activation of HIV-2 and HIV-1 LTR-directed gene expression were investigated. Like HIV-1 Tat, HIV-2 Tat contains conserved cysteine- and arginine-rich domains important for its function. However, HIV-2 Tat differs from HIV-1 Tat in that about 20% of the HIV-2 Tat at the amino terminus was not essential for its trans-activation function while HIV-1 Tat amino terminus is reportedly a part of its activation domain. Similarly, about 30% of the protein at the carboxy terminus of HIV-2 Tat was not essential. A domain critical for HIV-2 Tat-mediated trans-activation was located just upstream of the cysteine-rich domain. This segment is predicted to adopt an alpha-helical conformation and also contains acidic amino acid residues; thus, it may resemble amphipathic helix-type activation domains found in some transcriptional factors. A region with predicted hydrophobic alpha-helical character located between the cysteine- and arginine-rich domains was also important for HIV-2 Tat function. HIV-2 Tat mutants that were analogs of HIV-1 Tat **trans-dominant negative** mutants did not display such a phenotype.

L26 ANSWER 98 OF 118 MEDLINE on STN
93372102. PubMed ID: 8364040. Perturbation of the carboxy terminus of HIV-1 **Rev** affects multimerization on the **Rev** responsive element. Daly T J; Rennert P; Lynch P; Barry J K; Dundas M; Rusche J R; Doten R C; Auer M; Farrington G K. (Repligen Corporation, Cambridge, Massachusetts 02139.) Biochemistry, (1993 Aug 31) Vol. 32, No. 34, pp. 8945-54. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Perturbations within the transactivation and carboxy-terminal domains of HIV-1 **Rev** were examined for effects on **Rev** responsive element (RRE) binding activities in vitro and biological activity in vivo. Binding affinities, specificities, and multimerization of the transactivation mutants M10 and **Rev**/Rex M10-16 on the RRE were equivalent to wild-type **Rev**. Substitution of the Rex transactivation domain within **Rev** resulted in the incorporation of an internal methionine residue which, when cleaved with CNBr and subsequently purified, produced a protein species (CNBr-**Rev**) unable to fully multimerize on the RRE. Instead, two discrete protein-dependent species were generated in the gel shift assay. Furthermore, CNBr-**Rev** was observed to bind to the RRE with high specificity and an equilibrium binding constant of 6 x 10(-10) M. A C-terminal **Rev** deletion mutant (**Rev** M9 delta 14) lacking amino acids 68-112 displayed identical RRE binding characteristics to the CNBr-**Rev** protein. This protein, which lacks both the activation and the C-terminal domains, was biologically inactive but maintained the ability to discriminate the RRE from nonspecific RNA. Deletion of amino acids 92-112 resulted in a **Rev** mutant (**Rev** M11 delta 14) which bound to the RRE with wild-type affinity and high specificity. This purified mutant was observed to be aberrant in multimerization activity on the RRE with reduced multimerization apparent in the gel shift assay. However, **Rev** M11 delta 14 possessed biological activity equivalent to wild-type **Rev** in a cell-based p24 ELISA assay. These results suggest that polymerization on the RRE is dispensable for **Rev** activity and that two monomeric **Rev** proteins bound to the RRE are sufficient for biological activity. Furthermore, in vivo experiments using the **Rev**/Rex chimeric mutant and the M10 **transdominant** mutant as well as in vitro dissociation rate studies with **Rev** M11 delta 14 and **Rev** M9 delta 14 suggest that the M9 through M11 domain of the protein may be involved in RRE-dependent specific **Rev** dimerization.

L26 ANSWER 99 OF 118 MEDLINE on STN
93309489. PubMed ID: 7686621. Mouse monoclonal antibodies recognizing the activation domain of HIV-1 **rev** transactivator. Kreusel J; Aepinus C; Lang S; Beninga J; Prenzel S; Fleckenstein B. (Institut fur klinische und molekulare Virologie, Universitat Erlangen-Nurnberg, Germany.) Molecular and cellular probes, (1993 Apr) Vol. 7, No. 2, pp. 111-9. Journal code: 8709751. ISSN: 0890-8508. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The **rev** protein of human immunodeficiency virus type 1 (HIV-1), a phosphoprotein of 20 kDa apparent molecular weight, is essential to target the mRNA for virion polypeptides into the cytoplasm. So far, at least four necessary functional domains have been assigned to the HIV-1 **rev** protein: (1) one for RNA binding; (2) a second for nuclear/nucleolar localization that may be indistinguishable from the RNA binding motif; (3) two domains for multimerization; and (4) a putative activation domain (AD) that is suppressed in trans by **dominant-negative** mutant **rev** protein. We report three IgG1 kappa mouse monoclonal antibodies (mabs) that were independently raised against **rev** protein expressed in Escherichia coli. Epitopes are mapped by immunoprecipitation and Western blot screening with